Library
JUL 23 1942

Volume 32 *Number 7*

133

Phytopathology

AN INTERNATIONAL JOURNAL

July 1942

Official Organ of

The American Phytopathological Society

$6.25 per year, Canada $6.00 per year, United States and dependencies $6.50 per year, elsewhere
Office of Publication
Corner North Queen Street and McGovern Avenue, Lancaster, Pennsylvania
Entered at the postoffice in Lancaster, Pennsylvania, as second-class matter

TABLE OF CONTENTS

Ivan Claude Jagger ..... Donald Reddick 549

The relative effect of environmental and genetic factors on growth types of Ustilago zeae ..... M. F. Kernkamp 554

Coacervates in physical and biological systems.
J. Dufrenoy and H. S. Reed 568

The transient feeding of root-knot nematode larvae ..... M. B. Linford 580

Studies on black stem of alfalfa caused by Ascochyta imperfecta.
Maurice L. Peterson and L. E. Melchers 590

Lipolytic activity of phytopathogenic bacteria determined by means of spirit blue agar and its taxonomic significance.
Mortimer P. Starr and Walter H. Burkholder 598

The broad ring-spot virus ..... James Johnson and Robert W. Fulton 605

The genus Cerebella cesati—its biologic status and use.
R. F. Langdon 613

A quantitative method for assay of tobacco-mosaic virus protein.
Vernon L. Frampton 618

A method of growing plants in water vapor to facilitate examination of roots ..... Walter Carter 623

Chloropicrin and ethylene dichloride for root-knot nematode control.
A. G. Newhall and F. L. Starr, Jr. 626

Strawberry leaf roll, a new disease.
G. H. Berkeley and A. G. Plakidas 631

Phytopathological Notes 634

Banded chlorosis, a transmissible disease of cherry.
S. M. Zeller and J. A. Milbrath

Apothecia of Sclerotina fructicola on peach in western Washington ..... Glenn A. Huber and Karl Baur

A simple technique for aseptic handling of media.
J. Arthur Herrick

A nonpathogenic buff-colored barley smut ..... Wayne M. Bever

Heterocaryosis and variability ..... H. N. Hansen

On the cause of the milo disease ..... L. E. Melchers

Rhizoctonia infection of cotton and symptoms accompanying the disease in plants beyond the seedling stage ..... David C. Neal

Olive anthracnose in the United States.
Rafael E. Pontis and H. N. Hansen

Mycological Nomenclature ..... G. R. Bisby

Book Reviews 646

Report of the 1942 annual meeting of the Southern Division of the American Phytopathological Society 647

IVAN CLAUDE JAGGER
1889–1939

DONALD REDDICK

The geological formations known as drumlins are a characteristic of the landscape along the southern border of Lake Ontario. Between these elongated hillocks were deep bogs which at the present time are intensively cultivated peat lands known throughout the region as muck. In a farm-

IVAN CLAUDE JAGGER

house at the base of one of these drumlins and overlooking a peat bog not many miles from Marion, New York, there was born on August 12, 1889, to Claude N. and Alvinette (Andrew) Jagger their first of four sons. He was named Ivan Claude.

The peat bog, which had supported a growth of mint of sufficient extent to support a still for the production of oil, was brought under tillage during the youth of Ivan; and on it was produced lettuce, celery, onions, carrots and other vegetable crops. Thus the technique of successful vegetable culture was ingrained by everyday observation and experience.

Early schooling was finished in 1907 after a course in the high school at Marion, a course devoid of science except for mathematics. It was at just this time that the State of New York had come to the support of higher education in agriculture by the expansion of a department of agriculture in Cornell University into a College of Agriculture with new buildings and an enlarged staff with L. H. Bailey as Dean. H. H. Whetzel came to the College in 1906, H. J. Webber in 1907 and B. M. Duggar in 1908.

On September 24, 1907, Ivan Jagger matriculated as a "regular" student in the College of Agriculture and undertook 19 hours of work including botany, chemistry, zoology, entomology, and two courses in mathematics. On the basis of excellent work, he was recommended for graduation at the end of the seventh semester in order to enter the Graduate School. The degree of B.S.A. was granted in 1911 and was conferred *in absentia.* Jagger was already in a field laboratory near Elmira, New York, engaged in a project for the improvement of the potato crop. The following year he undertook work on an Industrial Fellowship to investigate the downy mildew of onions, caused by *Peronospora destructor;* but when the disease failed to appear his time was fully occupied with a lettuce disease, the leafspot diseases of celery, with the smut of onions and a variety of other diseases of vegetable crops. The first two studies ultimately led to publications with the description of *Sclerotinia minor* and of *Pseudomonas apii* [*Phytomonas jaggeri* (Stapp) Magrou]. The work with *Urocystis cepulae,* the smut of onions, led to interesting developments and these were to be incorporated in a thesis to be presented in partial fulfillment of the requirements for a Doctor's degree. The preliminary work on this thesis was used as the basis for a Master's degree, which was granted at the University of Wisconsin in 1913 following a year of work there, but a permanent record in the form of a thesis was not made. In the spring of 1914 Jagger suffered a severe attack of pneumonia, which, coupled with severe chronic asthma, prostrated him for several months. The dissertation was never written and the studies of the smut organism are lost. The disease was just beginning to appear in these newer areas and by his timely work on smut control, Jagger unquestionably made it possible for thousands of growers to preserve their land for onion culture with negligible losses and at trifling expense. All of his work with onions is reported in 204 words in an Extension Bulletin treating of the diseases of vegetables, a bulletin which was compiled with much conscientious effort as a distasteful duty.

The studies of *Urocystis cepulae* were put aside for, in the meantime, July 1, 1914, Jagger had been appointed assistant professor in the College of Agriculture at Cornell and instructor in Biology at the University of Rochester, a cooperative endeavor by the two institutions in an attempt to see whether something could be done about the diseases of vegetables at Irondequoit, the vegetable garden for the city of Rochester. The "curl" of cucumbers was soon demonstrated to be a communicable disease and not a case of malnutrition, as had been supposed. His research was confined

largely to the mosaic diseases of cucurbits, but his knowledge of diseases of vegetables and particularly his knowledge of how to be helpful without being obtrusive did a great deal to improve the plant sanitary conditions in this area of intensive crop production.

The conditions for work at Rochester were ideal in respect of research facilities, as well as the physical requirements of a chronic asthmatic, but in June, 1917, Jagger joined W. A. Orton's small army of inspectors and crop protectionists and was promptly sent to Florida where help was needed in the control of celery and lettuce diseases. It did not take long to develop a system of treatment which greatly improved the quality of the celery and the method is still employed in that area. Lettuce mosaic was investigated and a brief report made.

The asthma persisted and at Orton's suggestion Jagger went to Southern California in 1922, particularly in the hope that the climate would be beneficial to his health, but specifically to investigate an unknown disease of lettuce which was threatening the infant industry in the Imperial Valley. Jagger's own account of this "brown blight" disease has appeared posthumously in a recent volume of PHYTOPATHOLOGY but the practical results of his work are seen in the 8,000 carloads of lettuce which annually have moved out of the Valley to markets all over the United States. Both hybridization and selection within existing lines yielded highly resistant sorts, but the selection process in the hands of an experienced lettuce grower gave the practical result desired in a remarkably short time. In 1926, seeds were available to producers of seed. The growers had clamored for seeds in 1924, but Jagger insisted on one more year of testing. The clamor was then carried to Washington where a congressman tried to obtain seeds from W. A. Orton. Orton sent a telegram to Jagger and got back the reply, "one more year of testing." And so it was.

Upon releasing the lettuce seeds to producers, Jagger felt justified in taking the first vacation he had had since entering the profession. The year 1926 was spent quietly and with singular physical comfort studying, as an International Education Board Fellow, principally in the glass houses in England. But when he returned to California it was obvious that the work he had already instituted on the development of lettuce varieties that were immune to the mildew disease, caused by *Bremia lactucae*, must be pushed with vigor else the expanding acreage devoted to lettuce culture might have to be abandoned entirely. Furthermore, the powdery mildew of melons, caused by *Erysiphe cichoracearum*, was becoming increasingly destructive, and required attention. In 1930, in response to an inquiry, Jagger wrote as follows: "Prospects of a trip East for me are far in the distance. We are breeding disease resistant melons and lettuce, two generations of lettuce and three generations of melons each year, on limited funds and assistance. That leaves no time for anything except breeding melons and lettuce." A mildew-immune lettuce acceptable to the trade was produced only to have a new biological race of the parasite appear and make further work

necessary. For the present the lettuce mildew is subjugated and at the time of his death the melon mildew appeared to be under control. The recent outbreak of mildew on melons strongly suggests the introduction of a new biologic race of this organism, and assuredly suggests the Jagger technique for overcoming it. It was Jagger's nonconforming isolate of *Colletotrichum lindemuthianum* that forced M. F. Barrus to further studies and the classic announcement that biologic races of this organism exist.

Ivan Jagger was a most congenial companion and no one ever could so much as question his absolute honesty and sincerity. Although he never held a teaching position, he was, in fact, very effective in the field of adult education. Just as he found time to give attention to dozens of minor diseases of vegetables, so in all of his experiments, he took opportunity to include some things which he considered to be demonstrations and to which he could lead interested growers. His contacts with his colleagues were relatively limited. During his student days he attended all of the meetings of our Society and made a contribution to the program. He also became a sustaining life member of the Society in those stressful days of 1916. He was a Fellow of the American Association for the Advancement of Science, a member of the Botanical Society of America, American Society for Horticultural Science, and a Fellow of the Natural History Society of San Diego. Much of his life was spent in a far corner of the country in a simple laboratory which he set up. Long trips by train in midwinter were a luxury with which he wisely dispensed, so that he was known intimately only by the few who happened also to be in the same far corner. Just as he was familiar with a great variety of diseases from personal study, so also he was familiar with the breeding possibilities of a number of plants. In his spare time and as a hobby he had collected and hybridized species of *Iris, Gladiolus, Brodiaea, Lycium*, and *Penstemon*. His knowledge of the wild progenitors of cultivated plants and of their relatives was very extensive, and his discussions of such topics came as a surprise to most of his friends.

Jagger had a most retentive memory. This stood him in good stead, for he disliked making and filing records. He knew the pedigrees of his selections without looking in the file and, if his successors would admit it, he almost certainly left selections for which no record exists. He also disliked the preparation of manuscript for publication. All of his articles are comparatively short, but they are direct, clear, concise, and adequate.

It is entirely characteristic that the portrait from which the engraving has been made is an enlargement from a small print made for passport purposes. It is a good likeness of fifteen years ago.

Death came at San Diego, California, on February 16, 1939. Surviving are his wife, Gertrude Fisher Jagger, two sons, Donald 1923–, and Paul 1932–, his father, and one brother.

PUBLICATIONS OF IVAN CLAUDE JAGGER

The small lettuce Sclerotinia, an undescribed species. Phytopath. **3**: 74. 1913. (Abstr.)

A bacterial leafspot disease of celery. Phytopath. **4**: 395. 1914. (Abstr.)

Diseases of vegetables. West. New York Hort. Soc. Proc. **60**: 140–143. 1915.

Rotting of greenhouse lettuce. West. New York Hort. Soc. Proc. **60**: 147–148. 1915.

Diseases of vegetables. In "The Vegetable Industry in New York State." New York State Dept. Agr. Bull. **70**: 1320–1340. 1915.

Vegetable diseases. West. New York Hort. Soc. Proc. **61**: 175–177. 1916.

Experiments with cucumber mosaic disease. Phytopath. **6**: 148–151. 1916.

Two transmissible mosaic diseases of cucumbers. Phytopath. **7**: 61. 1917. (Abstr.)

Control of vegetable diseases. Cornell Univ. Agr. Exp. Sta. Ext. Bull. **19**: 559–580. 1917.

Hosts of the white pickle mosaic disease of cucumber. Phytopath. **8**: 32–33. 1918.

Mosaic diseases of cucurbits. Phytopath. **8**: 74–75. 1918. (Abstr.)

Some *Verticillium* diseases. Phytopath. **8**: 15–19. 1918. (Abstr., p. 75.) (With V. B. Stewart.)

Sclerotinia minor n. sp. the cause of a decay of lettuce, celery and other crops. Jour. Agr. Res. [U.S.] **20**: 331–334. 1920.

A transmissible mosaic disease of lettuce. Jour. Agr. Res. [U.S.] **20**: 737–740. 1921.

Bacterial leafspot disease of celery. Jour. Agr. Res. [U.S.] **21**: 185–188. 1921.

Immunity to mildew (*Bremia lactucae* Reg.) and its inheritance in lettuce. Phytopath. **14**: 122. 1924. (Abstr.)

Powdery mildew of muskmelons in the Imperial Valley of California in 1925. Phytopath. **16**: 1009–1010. 1926.

The brown blight disease of lettuce. Phytopath. **18**: 949–950. 1928. (Abstr.)

Disease resistant lettuce strains. Western Grower and Shipper. April, 1930. pp. 9, 22–26.

Melons resistant to powdery mildew. Phytopath. **21**: 113–114. 1931. (Abstr.) (With G. W. Scott.)

Lettuce breeding for disease resistance progresses rapidly. U.S.D.A. Yearbook **1931**: 348–350.

And now Imperial No. 13. Western Grower and Shipper. April, 1932. pp. 5–6.

Mildew resistant cantaloupes. Western Grower and Shipper. August, 1932. pp. 5–6, 22. (With G. W. Scott.)

Physiologic forms of *Bremia lactucae* on lettuce. Phytopath. **23**: 18. 1933. (Abstr.) (With Norman Chandler.)

Big vein, a disease of lettuce. Phytopath. **24**: 1253–1256. 1934.

Development of powdery mildew resistant cantaloupe No. 45. U.S.D.A. Circ. 441. p. 5. 1937. (With G. W. Scott.)

Breeding and improvement of cucurbits. U.S.D.A. Yearbook **1937**: 207–232. (With T. W. Whitaker.)

A new biologic form of powdery mildew on muskmelons in the Imperial Valley of California. U. S. Dept. Agr. Plant Dis. Reporter **22**: 275–276. 1938. (With T. W. Whitaker and D. R. Porter.)

Inheritance in *Cucumis melo* of resistance to powdery mildew (*Erysiphe cichoracearum*). Phytopath. **28**: 671. 1938. (Abstr.) (With T. W. Whitaker and D. R. Porter.)

Brown blight of lettuce. Phytopath. **30**: 53–64. 1939.

Cytogenetic observations in *Lactuca*. Jour. Agr. Res. **58**: 297–306. 1939. (With T. W. Whitaker.)

The inheritance of immunity to mildew (*Bremia lactucae*) in lettuce. Abst. in Proc. Seventh Internatl. Congress of Genetics. Phytopath. **30**: 427–433. 1940. (With T. W. Whitaker.)

The Imperial strains of lettuce. U. S. Dept. Agr. Circ. 596. p. 14. 1941. (With T. W. Whitaker, J. J. Uselman, and Walter M. Owen.)

THE RELATIVE EFFECT OF ENVIRONMENTAL AND GENETIC FACTORS ON GROWTH TYPES OF USTILAGO ZEAE[1]

M. F. KERNKAMP[2]

(Accepted for publication October 20, 1941)

When chlamydospores of *Ustilago zeae* (Beck.) Unger germinate, they ordinarily produce sporidia on their promycelia, but sometimes hyphal branches are formed instead of sporidia. The sporidia are easily isolated with a micromanipulator and, if allowed to grow vegetatively, will produce monosporidial lines. Many such lines have been isolated for the purpose of studying variability in this fungus. A phenomenon that complicates the study of variability in monosporidial lines of *U. zeae*, however, is the subsequent growth types of the resulting colonies. Unless hyphal branches are cut off, all colonies begin as single sporidia. The subsequent colonies may be entirely sporidial, entirely mycelial, or they may contain different proportions of sporidia and mycelium.

In a small percentage of cases the monosporidial isolates will continue to bud indefinitely and form colonies consisting entirely of sporidia. Such lines are designated as sporidial lines. If hyphal branches are cut off, they will produce colonies consisting of mycelium on potato-dextrose agar containing 1.5 per cent dextrose; but even some of these will form many sporidia on potato-dextrose agar containing 20 per cent dextrose. The lines that form no sporidia on agar with a 20 per cent sugar content are designated as mycelial lines. Since, however, strictly sporidial and mycelial lines occur very rarely, more attention will be given to those lines that change from a sporidial to an intermediate type of growth.

The change usually occurs during the first two months the lines are in culture, sometimes within the first 24 hours, but occasionally they change even after being in culture a year or more. The change generally occurs gradually, first becoming evident by the appearance of mycelial patches or pie-shaped mycelial sectors in the colonies. Several of these patches or sectors may appear simultaneously and thus all look alike, or they may appear at different times and, because of their different ages, look entirely different from each other. The question then immediately arises as to whether the latter constitute a genetic variation or whether the change from sporidial to an intermediate type of growth is temporary and phenotypic.

During studies on the variability of *Ustilago zeae* in the laboratories of the Division of Plant Pathology, University of Minnesota, many such

[1] Summary of a thesis presented in partial fulfillment of the requirements for the degree Doctor of Philosophy, granted by the University of Minnesota, June, 1941.

Paper No. 1926 of the Minnesota Scientific Journal Series.

Assistance in the preparation of these materials was furnished by the personnel of the Work Projects Administration, Official Project No. 65–1–71–140, sponsored by the University of Minnesota, 1941.

[2] Assistant Pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, U. S. Department of Agriculture. Formerly, Instructor, Division of Plant Pathology and Agricultural Botany, University of Minnesota.

patches and sectors have been isolated and compared in order to determine if they were genetic variants or only phenotypic changes. In many cases they have been found to be the latter. These circumstances made it confusing and often impossible to determine the degree of variability of monosporidial lines, and it seemed reasonable that if the factors that determine these growth types were better known, one could distinguish between phenotypic and genotypic changes with a greater degree of safety and thus make more accurate conclusions regarding the variability of monosporidial lines. Thus a study was begun on the factors that determine the growth types of monosporidial lines of *U. zeae*.

In a recent paper on genetic and environmetal factors affecting growth types in *Ustilago zeae* (6), the following conclusions were drawn: (1) Growth types are genetically inherited; (2) strictly sporidial and mycelial lines probably cannot be changed by environmental factors; (3) intermediate lines can be shifted to mycelial or sporidial by certain environmental factors. The results also proved that these growth types are determined by at least more than one factor; and it was assumed that sporidial and mycelial lines carried factors only for sporidial and mycelial growth, respectively, and intermediate lines carried factors for both sporidial and mycelial growth.

In that study, however, only a few monosporidial lines were studied and relatively few environmental factors were taken into consideration. It seemed desirable, therefore, to investigate more thoroughly the environmental factors that might change intermediate lines, and to determine if sporidial and mycelial lines could be changed by environmental factors not previously considered. This paper, therefore, presents the results of a continuation of previous work on the relative effect of genetic and environmental factors affecting growth types of *Ustilago zeae*.

The literature on this subject has been reviewed adequately in a recent paper (6) and will not be repeated here. Since literature on specific phases of the problem is not extensive, it will be referred to where pertinent.

EXPERIMENTAL PROCEDURE AND RESULTS

Methods

All of the lines used in these experiments, except those from cross 50, originated from single sporidia isolated from promycelia by means of a micromanipulator, as described by Dickinson (3) and Hanna (4). The lines from cross 50 originated from hyphal branches cut from promycelia with a micro-razor.

It was necessary to use lines relatively constant for their particular type of growth. Lines, therefore, that had been sporidial, mycelial, or intermediate for several months, and, in some cases, years, were selected from a large number of stock cultures. Many intermediate lines contain sporidia but appear to be mycelial to the naked eye. In order, therefore, to know accurately the growth types of all of the lines it was sometimes

necessary to examine them with a microscope. Having previously found that a high percentage of dextrose in the medium tended to increase formation of sporidia in intermediate lines (6), many of them were tested on potato-dextrose agar containing 20 per cent dextrose. Under these conditions one can distinguish between lines that will produce sporidia only with difficulty and lines that will not produce sporidia. The lines selected included 16 sporidial and 6 intermediate lines, and 1 mycelial line.

The designation of monosporidial lines indicates their origin. Thus, $70C_3$ is a monosporidial line isolated from the third cell of the promycelium of chlamydospore C of cross 70. Lines originating from hyphal branches were random isolates in that the chlamydospore and promycelial cell from which they came were unknown. Therefore, they were labelled with the cross number and a number indicating the order in which they were isolated. Thus, 50–2 is a culture which originated from the second hyphal branch isolate from cross 50.

The studies on the effects of environmental factors on the growth types of monosporidial lines were made with liquid media in hanging-drop cultures, with solid media in the form of agar smears on inverted cover slips, and with solid media in Erlenmeyer flasks.

In some cases a synthetic nutrient solution similar to that recommended by Brown (2) was used. It contained 20 g. dextrose, 2.5 g. asparagin, 2.5 g. tri-basic potassium phosphate, 0.2 g. magnesium sulphate, and water was added to make 1000 cc. This solution was varied so that different concentrations of dextrose were used, and where special substances were needed they were added in different concentrations to the basic medium. In the case of hanging-drop cultures a drop of each solution to be used was placed on each cover slip, which, in turn, was placed on a van Tieghem cell in a Petri dish, where the drops were kept from evaporating by means of filter paper placed on the bottom of the dish and moistened with distilled water. Five or 6 such drops were placed in each Petri dish, providing for as many replications.

When cultures were grown on solid media, potato-dextrose agar (1 or 2 per cent dextrose, 1.5 per cent agar, and infusion of 300 g. of potatoes for 1 liter of medium) was used in most cases. If agar drop cultures were employed, the procedure was the same as that described for the liquid hanging drops; if flasks were used, the cultures were grown in duplicate 250 cc. Erlenmeyer flasks containing 40 cc. of agar.

All similar media were prepared in one batch so as to have comparable media for each experiment. Each medium was prepared and sterilized in the autoclave for 20 minutes at 15 lb. pressure. In some cases special substances were added aseptically to the basic medium after it was sterilized.

EFFECTS OF ENVIRONMENTAL FACTORS ON GROWTH TYPES

Effects of Dextrose

Earlier experiments (6) had demonstrated that different amounts of

dextrose do not influence the growth types of sporidial and mycelial lines of *Ustilago zeae*, but that intermediate lines can be made to produce greater numbers of sporidia on media containing high concentrations of dextrose and fewer sporidia on media containing low concentrations of dextrose. The cultures used in the earlier study were of lines that had been grown long enough to become stable so far as their growth types were concerned, but it seemed desirable to study some lines that were changing. Therefore, lines were obtained that had been isolated less than 1 month and were changing from sporidial to an intermediate type of growth in their first cultural generation. These cultures had patches of both sporidial and mycelial growth in the same colony, and transfers were made from both the mycelial and sporidial patches. The resulting cultures were treated exactly alike, and their labels were followed by (M) or (S) to indicate if they had originated from the mycelial or sporidial part of the colony. The following lines were obtained in this manner: $48H_2$(M), $48H_2$(S), $48C_4$(M), $48C_4$(S), $46F_1$(M), $46F_1$(S), $46T_4$(M), and $46T_4$(S).

The above lines were grown in hanging-drop cultures in the liquid synthetic medium containing 0, 20, 100 g. of dextrose per liter. Each culture was replicated 6 times and the experiment was repeated twice. The results are recorded in table 1. From the table it is clear that none of the cultures behaved exactly alike in these solutions, but the general trend of behavior was similar in all. All of them produced more sporidia in the solutions containing the largest amounts of dextrose. The cultures that began as mycelium, excepting $46T_4$(M), first formed some sporidia, and after different periods ceased to form sporidia and again became mycelial. Isolate $46T_4$(M) did not form sporidia even in the solution containing 100 grams of dextrose. In each case it took longer for the growth types to revert to mycelium on the higher than on the lower dextrose concentrations. The lines that began as sporidia generally behaved alike, except that more sporidia were formed in them than in those commencing as mycelium. One became entirely mycelial in the solution without dextrose, and some did not form any mycelium in the solutions containing a greater amount of dextrose.

A similar experiment was made with the same lines and the same medium, except that agar was added to make it solid. The results of the latter experiment were generally the same as the former in that larger numbers of sporidia consistently were formed on media with a high concentration of dextrose.

Since hanging-drop cultures do not become large enough to express such cultural characters as appear in flask cultures, it is impossible to detect any variations in color, topography, and types of margins resulting from mutations. Thus, there still is the possibility that the change in growth types observed in hanging-drop cultures may be attributable to undetectable mutations. These same lines, therefore, were grown in duplicate 250-cc. Erlenmeyer flasks on agar, in order to let their cultural characters

TABLE 1.—*The growth types of sporidial and mycelial parts of colonies of intermediate haploid lines of Ustilago zeae after growing for various periods in hanging-drop solutions containing different amounts of dextrose*

Monosporidial line	Amount of dextrose in medium																					
	None										2 per cent								10 per cent			
	Hours										Hours								Hours			
	12		48		60		72		96		50		72		80		96		83		108	
	M[a]	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S	M	S
$48H_2$(M)	+[b]	+	+	+	+	tr	+	0	+	0	+	+	+	tr	+	0	+	0	+	0	+	0
$48H_2$(S)	0	+	+	+	+	tr	+	0	+	0	0	+	0	+	0	+	0	+	0	+	0	+
$48C_4$(M)	+	+	+	0	+	0	+	0	+	0	+	+	+	+	+	tr	+	0	+	+	+	0
$48C_4$(S)	0	+	0	+	0	+	tr	+	+	+	0	+	tr	+	+	tr	+	0	0	+	0	+
$46F_1$(M)	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0
$46F_1$(S)	+	+	tr	+	tr	+	tr	+	tr	+	0	+	0	+	0	+	0	+	0	+	0	±
$46T_4$(M)	+	0	+	0	+	0	+	0	+	0	+	0	+	0	+	0	±	0	+	0	+	0
$46T_4$(S)	+	+	+	+	tr	+	tr	+	tr	+	+	+	tr	+	tr	+	tr	+	0	+	0	+

Key: [a] M = mycelium
S = sporidial
[b] + indicates presence
0 indicates absence
tr = trace

develop fully. Potato-dextrose agar was used, containing 0, 20, and 200 grams of dextrose. The cultures were allowed to grow approximately 6 weeks after transfer to flasks; then they were examined both macroscopically and microscopically.

In this experiment the behavior of these lines was likewise generally the same as in the liquid hanging-drops and on agar drops. Generally there was more sporidial growth in the colonies on the media containing high concentrations of dextrose, and the colonies that began as sporidia all retained their sporidial growth on the medium containing 200 g. of dextrose per liter. To determine the permanence of the changes in growth type, reciprocal transfers were made from the 20 per cent to the 2 per cent dextrose medium, and from the 2 per cent to the 20 per cent dextrose medium. In all cases the cultures reverted to their original type and expressed the characters that appeared on the medium on which they were grown first. These results, therefore, indicate that mutations are not necessarily the causes of changes in growth types, since mutations are permanent changes. If the changes were due to mutations, it is not likely that the cultures would revert to the characters expressed on the original medium.

Effects of Extracts from "Natural" Substrates

Since the work of Brefeld (1), it has generally been accepted that the corn smut fungus will grow on such substrates as manure, soil, silage, and corn stalks, and that on these substrates aerial conidia are formed, which may be disseminated by wind. The writer attempted to determine to what extent soil, silage, manure, and corn extracts influenced the growth types of monosporidial lines of *Ustilago zeae*.

One mycelial, one sporidial, and several intermediate monosporidial lines were tested in hanging-drop cultures of these decoctions. Each culture was replicated six times and each experiment was repeated two or three times. Potato-dextrose broth was used as a check. The extracts were made sterile by filtering through a Sintered glass filter.

Soil Extract. Soil was obtained from the field plots at University Farm. One hundred cc. of distilled water and 100 g. of soil were mixed and allowed to stand so that the soil settled to the bottom. Then the water was poured off and filtered.

The soil extracts had no effect on the sporidial and mycelial lines, but almost completely prohibited sporidial growth in the intermediate lines tested. In potato-dextrose broth the intermediate lines were practically all sporidial growth. Since it is known that moisture stimulates sporidial growth (6), and soil extracts are obviously more liquid than soil, these results indicate that sporidia would not be formed in great numbers in soil in the field, except possibly in the case of strictly sporidial lines. Consequently, this would tend to decrease the possibility of dissemination of sporidia from soil by wind, but it would not decrease the possibility of the dissemination of aerial conidia.

Silage Extract. Piemeisel (10) reported that *Ustilago zeae* grew and formed sporidia in silage extract. In the writer's experiments, a silage extract was prepared as follows: 200 g. of silage, ground with a food chopper, were mixed with 200 g. of distilled water. After soaking for several hours the juice was expressed and filtered. This solution proved too concentrated to support growth, so it was diluted to one third of its original strength.

The same 3 haploid lines were grown in this solution; the mycelial line produced no sporidia; the sporidial line produced no mycelium; and the intermediate line became almost entirely mycelial. On potato-dextrose broth, the intermediate line was almost half sporidial and half mycelial. These results indicate also that if the fungus does grow on silage, only strictly sporidial lines would produce sporidia thereon.

Manure Extract. Fresh horse dung was obtained from the barns at University Farm. Equal quantities of manure and distilled water were mixed thoroughly; the resultant liquid was then expressed and filtered, and haploid lines of *Ustilago zeae* were grown in hanging-drops of the solution.

The growth types of the sporidial and mycelial lines remained unchanged in the manure extract. Five intermediate lines tested were entirely mycelial in the manure extract, but produced many sporidia in potato-dextrose broth. Another intermediate line produced a few sporidia in the manure extract, but in potato-dextrose broth it became almost all sporidial.

Corn Extract. It is obvious that *Ustilago zeae* forms hyphae in the host plant. Even lines that ordinarily form mostly sporidia in culture will form mycelium when injected into the host plants. However, several strictly sporidial lines have been found that have never caused infection in corn. It was, therefore, considered that these lines probably had no factors for mycelium formation, even in the host. It seemed desirable to determine if corn juice would change sporidial lines to mycelial, and, furthermore, if it would have any effect on intermediate and mycelial lines.

Fourteen-day-old corn seedlings were ground up with a food chopper and the juice was pressed from them and filtered. Hanging-drop cultures of a sporidial line, 2 intermediate lines and a mycelial line were grown in the extract. In the corn juice, the intermediate lines were almost entirely mycelial, while in the potato-dextrose broth, they were only approximately half-mycelial. In the sporidial and mycelial lines there was no apparent difference between growth in corn juice and in checks.

Another experiment was made in which corn juice was sterilized by autoclaving. In the autoclaved solution, there was slightly more sporidial growth in the intermediate lines than when they were grown in the filtered solution, but this solution had no effect on the mycelial and sporidial lines.

These results demonstrate clearly that corn juice stimulates mycelial growth in the intermediate lines, and add further evidence to indicate that strictly sporidial lines will not form mycelium, even in the host.

The results obtained from growing these lines on natural extracts may

serve to indicate how intermediate lines behave in nature. Since nearly all lines are intermediate, it follows that most of them may behave as these results indicate. It is possible, however, that other soil organisms and various combinations of environmental factors in the soil may influence growth types. If few sporidia are formed on the substances tested, there obviously will be less dissemination of sporidia in the field from such sources as manure piles, corn stalks, and soil. This, of course, does not prevent spread of spores carried on manure or soil blown about by the wind, but it would decrease possible multiplication of sporidia and their dissemination from these sources.

Effect of Vitamin B_1 and "Growth Promoting" Substances on Growth Types

It has been reported by Schopfer and Blumer (11) that *Ustilago zeae* grows perfectly well on nutrient media without Vitamin B_1. Either the fungus does not require this vitamin, or it synthesizes it from the chemicals in the medium. However, it was considered by the writer that Vitamin B_1 might have some influence on the growth types of *U. zeae*, even though it is unnecessary for the nutrition of this fungus in synthetic media.

An experiment was made in which monosporidial lines of *Ustilago zeae* were grown in synthetic media containing 3, 30, 300, and 30,000 units of Vitamin B_1 per liter, but in no case were the growth types of the lines influenced by this Vitamin.

Along with vitamin B_1, two "growth promoting" substances, indole-3-n-propionic acid and tryptophane, were tested and found to have no effect on the growth types of *Ustilago zeae*.

Effect of Poisons on Growth Types

Earlier results (6) have indicated that conditions unfavorable to growth of *Ustilago zeae* tend to initiate in culture the formation of mycelium instead of sporidia. For example, an excess of magnesium sulphate in the medium decreased the rate of growth, as well as prevented the formation of sporidia, in intermediate lines. Such behavior was attributed to the toxic effects of the various chemicals when present in excessive amounts.

To test the validity of such an assumption an experiment was made in which the behavior of a sporidial, a mycelial, and 2 intermediate lines of *Ustilago zeae* were grown in nutrient solutions containing mercuric chloride, copper sulphate, lead acetate, and iron chloride. These chemicals were added to the basic solution mentioned earlier in the paper in the proportions of 1:500, 1:1000, 1:2000, and 1:5000, the basic solution without poisons being the check. Each culture was replicated 5 times and the experiment was repeated 3 times.

The mycelial and sporidial lines did not change when tested with the solutions containing toxic chemicals. The intermediate lines, however, produced fewer sporidia as the concentrations of the poisons were increased,

and generally became entirely mycelial in the stronger solutions after 24 to 36 hours, and in the weaker solutions after 60 hours. There was little difference between the checks and the cultures in the solutions with the weakest dilutions of the poisons, but there was a striking difference between the checks and the cultures in solutions with high concentrations of poisons. These results, however, were somewhat erratic. For example, in the tests with copper sulphate at all concentrations, and with lead acetate 1 : 500, the intermediate haploid lines produced more sporidia when the cultures were approximately 24 hours old, and when they became 50 to 60 hours old they formed more mycelium and fewer sporidia. At 60 hours many of the sporidia became granular, stopped growing, and, consequently, did not germinate to form mycelium. As a result these cultures still contained many sporidia at the time growth ceased; therefore, they could not be considered truly mycelial at the termination of the experiment, as had been true with the other cultures. Nevertheless, it was clear that many sporidia were formed at the beginning of the experiment and, near the end of the experiment, all new growth was mycelial.

The results, as a whole, seemed to justify the general conclusion that toxic materials limit the formation of sporidia and increase proportionately the formation of mycelium in intermediate monosporidial lines of *Ustilago zeae*.

Effect of Dyes on Growth Types

Many organic dyes are toxic to fungi and in some cases are recommended for the control of certain plant diseases (9). Therefore, along with inorganic poisons, it seemed desirable to determine the effect of several dyes on the growth types of sporidial, mycelial, and intermediate lines of *Ustilago zeae*.

For this experiment malachite green, brilliant green, and crystal violet were added to potato-dextrose broth in the proportions of 1 : 1,000,000, 1 : 2,500,000, and 1 : 5,000,000. One sporidial line, 1 mycelial line, and 2 intermediate lines were tested. Each culture was replicated 6 times, and the experiment was repeated twice, potato-dextrose broth without dye being the check.

None of the dyes influenced the growth types of the sporidial and mycelial lines, and brilliant green did not appear to influence the growth types of the intermediate lines. The other two dyes limited the formation of sporidia in the stronger solutions. Malachite green was the most effective, and there were fewer sporidia in the 1 : 1,000,000 malachite green solution than in the lower concentrations. Brilliant green 1 : 1,000,000 appeared to be the only concentration of that dye that stimulated the formation of mycelium in the intermediate lines, growth in the other 2 concentrations being similar to that in the check.

Effect of Other Organic Compounds on Growth Types

Among chemicals tested for their therapeutic value in controlling plant

diseases, para-toluene-sulfonylamide and ortho-toluene-sulfonylamide appear to be most promising (5). The effect of these two chemicals on the growth types of monosporidial lines of *Ustilago zeae* was tested.

In these experiments a sporidial, a mycelial, and an intermediate line of *Ustilago zeae* were grown in hanging-drop cultures. The nutrient solution consisted of these chemicals added to the basic synthetic solution in the proportions of 1:1000, 1:10,000, 1:100,000, and 1:1,000,000. Each culture was replicated five times, and the experiment was repeated twice. The basic solution with nothing added was the check.

Para-toluene-sulfonylamide and ortho-toluene-sulfonylamide had no effect on the growth type of any of the lines tested in these experiments, but there seemed to be some influence on the rate of growth. These chemicals at the concentration of 1:1000 definitely retarded the growth of the fungus, and ortho-toluene-sulfonylamide 1:1,000,000 seemed slightly to accelerate the rate of growth over the checks.

Effect of Carbon Dioxide and Oxygen Supply on Growth Types

It has generally been observed that when *Ustilago zeae* grows beneath the surface of liquid nutrient media it does not form sporidia, but the growth is characterized by narrow hyphae. Furthermore, if sporidia are formed in cultures growing in liquid media, they are invariably produced on the surface of the solutions. The oxygen supply in the solution and on the surface has generally been assumed to be the main factor involved in bringing about these different growth forms. Experiments, therefore, were made to test the effect of carbon dioxide and oxygen supply on the growth types of monosporidial lines of *U. zeae*.

The lines of *Ustilago zeae* were grown in hanging-drops of potato-dextrose broth, which were placed in atmospheres of approximately 50 per cent oxygen and 50 per cent air, 50 per cent carbon dioxide and 50 per cent air, 75 per cent oxygen and 25 per cent air, and 75 per cent carbon dioxide and 25 per cent air. Cultures grown only in air served as checks. As indicated in table 2, the growth types of the sporidial and mycelial lines were not changed by the different atmospheres of carbon dioxide and oxygen, but the rate of growth in the atmospheres containing 50 per cent and 75 per cent carbon dioxide was considerably reduced. A high carbon dioxide content in the atmosphere did not change the growth types of the intermediate lines but did retard the rate of growth. A high oxygen content of the atmosphere increased the amount of sporidial formation in the intermediate lines.

Effect of Osmotic Concentrations of Nutrient Solutions on Growth Types

There are a number of species of algae that have 2 distinct growth forms depending upon the culture media in which they are grown. One form is characterized by spherical cells that separate from each other after cell division; the other is a filamentous form. Livingston (7) reported in his

experiments with a species of *Stigeoclonium*, that nutrient solutions of high osmotic concentration stimulated the spherical-cell form, and nutrient solutions of low osmotic concentration stimulated the filamentous form.

One can readily see the similarity between the growth forms of this species of *Stigeoclonium* and *Ustilago zeae*, and it seemed reasonable to suppose that similar reactions might occur in *U. zeae*. Although the results reported earlier in this paper indicate that changes in growth types are stimulated by the quantity or availability of the various food elements used,

TABLE 2.—*The growth types of sporidial, mycelial, and intermediate haploid lines of Ustilago zeae in atmospheres containing proportions of carbon dioxide and oxygen to air*

Monosporidial line	50% CO_2		75% CO_2		50% O_2		75% O_2	
	M[a]	S	M	S	M	S	M	S
$18C_3$ (sporidial)	0[b]	+	0	+	0	+	0	+
50–2 (mycelial)	+	0	+	0	+	0	+	0
$22C_1$ (intermediate)	+	+	+	+	+	+	tr	+
$22B_2$ do	+	+	+	+	±	+	tr	+
$70C_3$ do	+	+	+	+	±	+	tr	+

Key: [a] M = mycelium
S = sporidial
[b] + indicates presence
– indicates absence
tr = trace
± approximately 25% mycelium and 75% sporidia in the cultures.

they did not preclude the possibility that the stimulating factor might be the osmotic concentration of the nutrient solutions. Experiments, therefore, were made to determine whether osmotic concentration of nutrient solutions would influence the growth types of *U. zeae*.

Livingston's experiments (7) were repeated, with only slight variation in the quantities of nutrients used, with *Ustilago zeae*. The osmotic concentrations of the solutions were calculated from the International Critical Tables of Freezing Point Depression of solutions, and checked by the freezing point method described by Loomis and Shull (8). Mycelial, sporidial, and intermediate lines were tested in solutions of different osmotic concentrations. In no case did these solutions influence the growth types of these lines. The experiment was repeated 3 times; the third time small but equal quantities of dextrose were added to the solutions. This only stimulated better growth; but, again, there was no difference in the growth types of any culture in the solutions of different osmotic concentrations.

EFFECTS OF GENETIC FACTORS ON GROWTH TYPES

Sporidial × Sporidial Matings

It has been mentioned previously that certain monosporidial lines have never formed mycelium in culture, either on potato-dextrose agar or under the different environmental conditions considered in these experiments.

Crosses had been made between some of these lines and mycelial lines, and there was segregation for sporidial and mycelial growth types in the progenies of these crosses (6). Likewise, successful crosses were made between sporidial and intermediate lines. Efforts were made to hybridize sporidial lines for the purpose of studying the inheritance of the sporidial character, but all attempts at hybridization were unsuccessful. Sixteen sporidial lines were paired in all possible combinations and injected into corn seedlings in the greenhouse, but in no case did infection result. This was repeated at least 10 times, but still no infection was obtained. The question immediately arose as to why these lines failed to cause infection when inoculated into corn seedlings. Two possible explanations for this behavior may be that all of the lines are of one sex and consequently sexually incompatible, or that the lines are so definitely sporidial that they cannot form hyphae in the host and, consequently, cannot fuse to form the dikaryotic mycelium.

The first possibility was eliminated by crossing some of the sporidial lines with tester lines of opposite sex. Table 3 gives the results of these matings.

TABLE 3.—*The compatibility of several sporidial lines with two tester lines of opposite sex*

Sporidial line	Tester lines of opposite sex	
	$22B_2$	$22B_4$
$18C_3$	+[a]	−
$18A_1$	−	+
$18H_1$	+	−
$18H_4$	+	−
$18A_4$	−	+

Key: [a] + = compatible
− = incompatible

Lines $22B_2$ and $22B_4$ are of opposite sex and cause infection readily. As is indicated in table 3, $18C_3$, $18H_1$, and $18H_4$ were all compatible with $22B_2$, and $18A_1$ and $18A_4$ were compatible with $22B_4$. Hence it follows that $18C_3$, $18H_1$, and $18H_4$ are of a different sex from $18A_1$ and $18A_4$. This was done also with several other pairs of monosporidial lines known to be compatible, and similar results were obtained.

The second possibility was tested by making histological studies of corn seedlings that had been inoculated with a composite inoculum of several strictly sporidial haploid lines. The seedlings were sectioned at the point of injection 24, 48, and 72 hours after the injections were made. The sections of the fresh tissue were stained with cotton blue or safranin and fast green. In the stained sections, numerous sporidia could be seen, but in no case were the sporidia observed to germinate and form hyphae. This was repeated many times and the results were always the same. Corn seedlings, inoculated with compatible haploid lines, were treated in the same manner, and mycelium invariably could be found in these plants.

It is possible, but hardly probable, that the mycelium could have been

overlooked in the plants inoculated with strictly sporidial lines. The results indicate that these sporidial lines will not form mycelium inside the host plant, and, consequently, cannot cause infection when mated with each other. However, the strictly sporidial character can be perpetuated by combining these lines with intermediate or mycelial lines. It seems reasonable that sporidial and mycelial lines may combine by the growth of hyphae of the mycelial or intermediate line, so that it comes in contact with the sporidia of the sporidial line within the corn plant, and in this way fusion can occur.

SUMMARY AND CONCLUSIONS

These results are in agreement with those reported earlier (6), namely, that growth types of monosporidial lines of *Ustilago zeae* may be strictly sporidial, strictly mycelial, or intermediate between sporidial and mycelial. The growth types of sporidial and mycelial lines cannot be changed, but the intermediate lines can be shifted one way or the other by various environmental factors tested. Dextrose was the most efficient in stimulating the formation of sporidia in intermediate lines. Mycelial formation in intermediate lines in general was stimulated by environmental conditions that were unfavorable to the growth of the organism. For example, the addition of poisons and toxic dyes to media, low concentrations of necessary nutrients, and of oxygen in the atmosphere stimulated the formation of mycelium. Other substances, such as vitamin B_1, indole-3-n-propionic acid, tryptophane, para-toluene-sulfonylamide, and ortho-toluene-sulfonylamide, had no effect on growth types.

The high concentrations of sugars in some of the media indicated that the formation of sporidia in intermediate lines might be brought about by the high osmotic concentrations of those media. This possibility, however, was eliminated by experiments proving that the osmotic concentration of nutrient media was not the stimulating factor. All of the results indicate that the quantity of nutrients in the media is the most important factor in determining the growth types in intermediate lines in culture. Although some of the other environmental factors influenced the growth types of intermediate lines to some extent, their effects were not nearly so strong as those of nutrients; in fact, lines, recently isolated, could be retained in the sporidial condition by growing them on media with a high sugar content. Furthermore, after lines had changed to an apparently mycelial growth type, the writer was able to change many of them back to intermediate, and thereby distinguish between those lines apparently entirely mycelial and those almost so, but producing a few sporidia. Since the change from sporidial to intermediate type often can be prevented or reversed by environmental conditions, these results would indicate that in many cases it is due to physiological processes.

The experiments with "natural" substrates serve to indicate how *Ustilago zeae* may behave in nature. All of the "natural" substrates definitely limited sporidial formation in intermediate lines and stimulated mycelial

formation. Virtually all lines of *U. zeae* are intermediate. Therefore, if these results do indicate what takes place in nature, it follows that most of the growth in nature would be in the form of mycelium. In this paper, however, no consideration was given to effects of other organisms in the soil or other soil factors, but it is highly probable that they might influence growth types also.

Stakman (12) reported clear-cut segregation for growth types in the promycelia of chlamydospores, and Kernkamp (6) reported segregation for growth types in the progeny of a cross between a sporidial and a mycelial line, thus definitely proving that growth types are inherited. Crosses between intermediate lines that are mostly sporidial have given rise to progenies that are mostly sporidial, and crosses between lines that are mostly mycelial have increased the prevalence of mycelial segregates in those progenies.[3] But efforts to cross strictly sporidial lines were always unsuccessful. After examining many sections of corn plants inoculated with combinations of sporidial lines and invariably failing to find mycelium in these plants, it was concluded that these lines could not cause infection when mated together because they are incapable of forming mycelium, even in the host. They are, however, pathogenic when mated with lines that form hyphae. This strengthens the general conclusion that the strictly sporidial growth types are limited by genetic factors and cannot be changed by environment.

DIVISION OF PLANT PATHOLOGY AND BOTANY,
AGRICULTURAL EXPERIMENT STATION,
UNIVERSITY FARM, ST. PAUL, MINNESOTA.

LITERATURE CITED

1. BREFELD, OSCAR. Untersuchungen aus dem Gesammtgebiete der Mykologie. Heft XIII. Die Brandpilze IV. 75 pp. Münster. 1905.
2. BROWN, WILLIAM. Studies on the genus Fusarium II. An analysis of factors which determine the growth forms of certain strains. Ann. Bot. **39**: 373–408. 1925.
3. DICKINSON, SIDNEY. A method of isolating and handling individual spores and bacteria. Proc. Roy. Soc. Med. London **19**: 1–4. 1926.
4. HANNA, W. F. A simple apparatus for isolating single spores. Phytopath. **18**: 1017–1021. 1928.
5. HART, HELEN, and J. LEWIS ALLISON. Toluene compounds to control plant diseases. Phytopath. **29**: 978–981. 1939.
6. KERNKAMP, M. F. Genetic and environmental factors affecting growth types of *Ustilago zeae*. Phytopath. **29**: 473–484. 1939.
7. LIVINGSTON, BURTON EDWARD. On the nature of the stimulus which causes the changes of form in polymorphic green algae. Bot. Gaz. **30**: 289–317. 1900.
8. LOOMIS, WALTER E., and CHARLES A. SHULL. Methods in Plant Physiology. McGraw-Hill Book Company, (New York). 472 pp. 1937.
9. MARTIN, HUBERT. The scientific principles of plant protection with special reference to chemical control. Edward Arnold and Co. (London). 385 pp. 1940.
10. PIEMEISEL, F. J. Factors affecting the parasitism of *Ustilago zeae*. Phytopath. **7**: 294–307. 1917.
11. SCHOPFER, W. H., and S. BLUMER. Les facteurs de croissance des espèces du genre Ustilago. Compt. Rend. Acad. Sci. Paris. **206**: 1141–1143. 1938. Abst. in Rev. Appl. Myc. 17: 618. 1938.
12. STAKMAN, E. C. The problem of specialization and variation in phytopathogenic fungi. Genetica **18**: 372–389. 1936.

[3] Unpublished data.

COACERVATES IN PHYSICAL AND BIOLOGICAL SYSTEMS[1,2]

J. Dufrenoy and H. S. Reed

(Accepted for publication March 17, 1942)

Our previous studies on the effects of a nutrient deficiency in orange leaves, known as "mottle-leaf," called attention to certain vacuolar inclusions not found in cells of healthy leaves (13, 14). These inclusions were generally refringent in transmitted light and each was surrounded by a distinct layer that would absorb dyes, such as acid fuchsin, and could be plainly seen under the microscope. In the absence of more exact information at that time, we concluded that these spherical bodies contained phytosterol material or phospho-lipoids. The affected orange leaf cells gave evidence that there had been a decided shift in the oxidation-reduction equilibrium. Cells of the affected leaves could also be distinguished by their power to reduce methylene blue and Nile blue A.

These spherical inclusions were not confined to the leaves, but we demonstrated them in the post-meristematic cells of the vegetative buds of orange shoots on affected branches (12). The enveloping membrane and the tendency to clump were there clearly demonstrated, though at the time of the publication no physico-chemical interpretation of the inclusions was given. However, in a later publication we advanced the idea that these spherical refringent bodies represent suboxidized products of the metabolism of carbohydrates and proteins in these affected cells. In the case of the zinc-deficient cells, there was evidence that the sulfhydryl compounds, such as cystein, were stabilized by the zinc salts that were present.

We have been able to demonstrate the presence of these inclusions in living cells by the application of neutral red or methylene blue in low concentrations; hence we assured ourselves that we were not dealing with artefacts. Moreover, other investigators, employing their own techniques, have described analogous bodies in the cells of other plants.

We shall in this paper present further investigations on the nature of these characteristic inclusions in pathological plants.

RECENT STUDIES

Since a knowledge of the factors that affect the stability of hydrophilous sols is important for pathologists and physiologists, we shall mention a few recent contributions to the subject dealing with non-living systems. It is known that under certain conditions there may appear, in an hydrophilous sol, bodies in which colloids have become concentrated through a phenomenon that might be termed a "demixing," or a separation of phases (9) or, more precisely, a "coacervation" process (7). The coacervate is a body rich in colloids and immersed in a liquid relatively poorer in colloids, which has been designated the equilibrium liquid.

[1] The work herewith reported was done in the Division of Plant Nutrition, University of California, Berkeley.

[2] Cost of publication of this paper was borne by the University of California.

Bungenberg de Jong and Kruyt (7) postulated that flocculation and coacervation are very closely allied phenomena, each requiring the elimination of the stability factors, charge and hydration. For example, in the presence of neutral salts, a polyphenol may induce either flocculation or coacervation, according to the temperature and viscosity (as determined by mucilages).

Coacervation results from a lowering of the "solvation" and a reduction of the charge on the particles that may become coacervated. To remain suspended or dispersed as an hydrophilous sol, each particle must be surrounded by its own "solvation mantle," meaning a peripheral zone of molecules of water in the solvation medium. The molecules of water nearest to the particle are oriented around it; farther away, the molecules are less and less distinctly oriented, so that they gradually become distributed at random in between the different "solvation mantles," as Bungenberg de Jong pointed out (6). The action of the desolvating agent reduces the large diffuse solvation mantle around each particle to a thin layer of oriented water molecules, which are sharply separated from the bulk of surrounding, randomly distributed water molecules. These oriented water molecules are surrounded by a boundary, which plays the role of an active layer. Following the diminution in thickness of the solvation mantle through the desolvation process, the particles may gather so closely that each layer of surrounding oriented molecules of water loses its individuality and the group of particles becomes coacervated within a common boundary which separates the "coacervate" as a whole from the surrounding equilibrium liquid. Hildebrand (5) concluded that one cannot make a definite distinction between the water acting as a solvent and that acting as water of hydration, since the solute is chemically bound to some of the water and this, in turn, chemically bound to water molecules farther removed.

First, we shall inquire what might happen if one would introduce into this structure solutes of different types—for example, ions with single or multiple charges; molecules with dipoles, or molecules, which, themselves, can participate in the formation of hydrogen bonds, because it seems logical to interpret a coacervate as the result of a disturbance of hydrogen bonds in the water solution through the agency of oxidases such as catechol oxidase. Second, we will investigate the process of vital staining, as a disturbance of hydrogen bonds through the introduction of electrically charged particles of the vital stains. Third, we shall interpret in the same light the significance of "hydrogen donors" as solvents of the coavervates.

Ethyl alcohol, methanol, acetyl acetone, acetone, and tertiary butyl alcohol were reported by Russell (15) to be good solvents of natural phlobatannins, which are understood to be oxidation and polymerization products of catechol. To these should be added ethylene glycol and dioxan (diether of glycol), methyl cellosolve, and tetra ethylene glycol dimethyl ether.

Among these solvents, those of special interest to us now are the "donor solvents," which are able to release hydrogen to the substance to be dissolved

(or solute), thereby building a solute-solvent association by means of CH ← O bonds. Hydrogen donors as solvents have been studied by Zellhoefer, Copley, and Marvel (16) and by Copley, Zellhoefer, and Marvel (2, 3).

The biological importance of the phenomenon becomes significant when we pay attention to the correlation between oxidation of catechol and polymerisation as oxidation processes, and to linkages that unite molecules, building up molecular associations that may ultimately reach microscopic sizes. These are reversible phenomena, and introducing H into the system will both induce reduction and break up the molecular linkages, as H will satisfy many of the bonds, and compete with molecular linkages. Therefore, it was to be expected that hydrogen donors would act as solvents of the oxidized, polymerized and coacervated catechol colloids.

Complex micellar systems are those in which complex relations between micelles exist; that is to say, those in which an effective attraction of charges is opposed to the tendency to solvation of the components. "Complex" and "auto-complex" coacervates as defined by de Jong (6) form only in the particular cases of these complex colloidal systems.

These are of two kinds:

(a) the opposition of the charges is created by the charged colloids of opposite signs = complex colloidal systems in a strict sense;

(b) the opposition of the charges is created by adsorption or linkage of appropriated ions at the surface of a single species of particular colloids = auto-complex colloidal systems.

Among the different types of coacervates that can be conceived as physically possible, it is advisable to reconsider Bungenberg de Jong's (6) "auto-complex," in which the coacervation involves phosphatides and is irreversible. He emphasized its ability to induce persisting desequilibria between adjoining solutions. As the movement from outside or from within of any given solute would be determined by the "permeability," auto-complex coacervates would contain the elements each of which had been considered as responsible for cell permeability—namely, water, molecules of cholesterol or phytosterol, and a certain number of adsorbed calcium ions.

The equilibrium between the coacervate and the surrounding liquid may be altered by further lowering the solvation. This would retard the diffusion of some of the solvate liquid out of the coacervate. It is not to be denied that the change of equilibrium may be so abrupt that it provides no time for diffusion. When that state exists in the system, some of the solvating agent will separate out as "vacuoles" within the coacervate. When those drops are exceedingly small and numerous, they cause the coacervate to appear black under the microscope. The writers frequently found this to be the case in walnut leaves showing the effects of a deficiency of zinc.

Many of the coacervates present a notable resistance to high salt concentrations. For example the concentration of NaCl reaches 200 milliequivalents for some and may rise to 0.8 N at the time when it does not exceed

50 m.e. in complex coacervates of gelatin with gum arabic, and 12 m.e. with those of gelatin with egg lecithin. This fact argues strongly in favor of the hypothesis assigning to the complex coacervates a part in the constitution of living matter.

According to Klercker (8) polyphenols may occur in solution in some plant cells; in others they may occur as characteristic tannin-vacuoles, each with an enveloping membrane. He described the complex coacervates formed in the vacuolar sap of plants when cells were submerged in a hypertonic solution of KNO_3. The cause of the phenomenon may be referred to the desolvating action of the salt solution, which withdrew water from the cell vacuole and led to coacervation of the catechols. When the KNO_3 was washed out there was a disappearance of the coacervates due to the dispersion of the catechol. It can be termed an increase of "solvation," *i.e.*, mixing.

Molisch (11), who investigated crystalline and amorphous anthocyanins, found with surprising frequency the occurrence of anthocyanins as crystals or as amorphous aggregations in the vacuolar sap. The latter, which were generally globular, had tendencies (as shown by illustrations) to zonation. Molisch tacitly assumed that the crystals or balls in such cases consisted of a chemical combination of the pure pigment with another substance, possibly a tannin compound.

Massee (10) saw and described "tannin-vesicles" in the vacuoles of leaf cells of orchids affected with the "spot" disease, explicitly stating, however, that the vesicles do not consist entirely of tannin. He believed that in many respects they resembled the tannin aggregates described by Klercker. The illustrations accompanying his article show several types of vacuolate globules, which occurred in the cells of affected orchid leaves.

At present there can be no doubt that the globular aggregates formerly seen in plant cells by various observers were complex coacervates of catechol and lipoid materials.

Coacervates are porous systems or molecular sieves, inasmuch as the micellae are separated by the solvatant liquid. Lecithin, phytosterol, or other bodies accumulated in the solvatant membrane, are lipoids and their role in permeability was postulated by Overton. Since the solvatant membrane contains water, as well as the accumulated lipoids, it, therefore, seems possible to consider it as a mosaic of water and lipoid constituents, as assumed by Collander (1).

The main factor making possible the auto-complex coacervation of a phosphatid is a certain stage of desolvation. This could be achieved through a water-soluble desolvant, such as catechol or other polyphenols, though without any permanence, as these water-soluble desolvants would immediately be dispersed in the surrounding liquid, but the living cell also contains desolvants, *e.g.*, phytosterol, which are practically insoluble in water, though able to enter into complexes with water. The cytological significance of phytosterol has been previously emphasized by Guilliermond and Mangenot.

CYTOLOGICAL INVESTIGATIONS

It is the purpose of the present paper to present some data obtained from the study of plant roots and to relate certain pathological aspects to the supply of mineral nutrients. We shall emphasize the abnormal organization of the root cells with especial reference to the presence of globular aggregates and discuss the processes of coacervation in relation to inclusions that occur in the cell vacuoles. The whole question of the relation of catechol to growth and cell physiology has taken on greater significance as a result of recent advances in the physical and the biological sciences.

Orange seedlings, grown for a time in a nutrient solution deficient in zinc, were characterized by hypertrophied root tips (Fig. 1) similar to tomato roots described by Eltinge and Reed (4). An inspection of

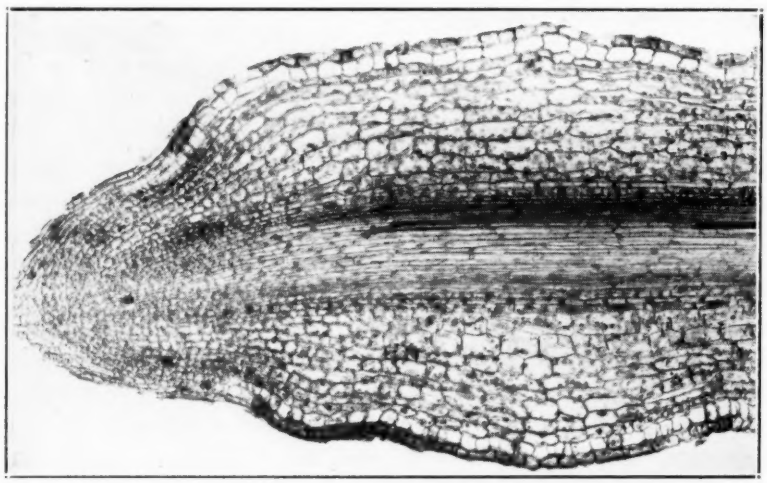

FIG. 1. Longisection of apical region of an orange root grown in a zinc-deficient nutrient solution. The cells of the periblem had been hypertrophied with the production of an abnormal swelling in the post-meristematic region.

figure 1 makes evident the connection between the macroscopic and microscopic features of the root. There was a strong tendency for the cortical cells to be enlarged radially in contrast with the lack of growth in the axial direction. The globular coacervates in the cells of these affected roots resembled in all essential ways the vacuolar inclusions that the writers found in orange leaves (12). The contrast between healthy and affected root cells is evident from the photomicrographs shown in figure 2, A and B. The former had grown for several months in a culture containing all the necessary microelements. Its postmeristematic cells were free from coacervates with the exception of a few cells near the surface, which were about to be cast off from the growing expanding root tip. The lower figure represents cells from the comparable region of an orange root tip that had grown in a nutrient solution containing all the necessary microelements, except zinc. The section shows the characteristic enlargement of many of the cells and

the almost universal presence of globular inclusions in the cells. From these the nucleus was easily defined through the use of the molybdenic reagent, which stains the chromocenter of the nucleus a deep blue, as molybdenum blue is being formed at the site of the nucleoproteins.

Figure 3, A, shows in greater detail the contents of healthy cells of the

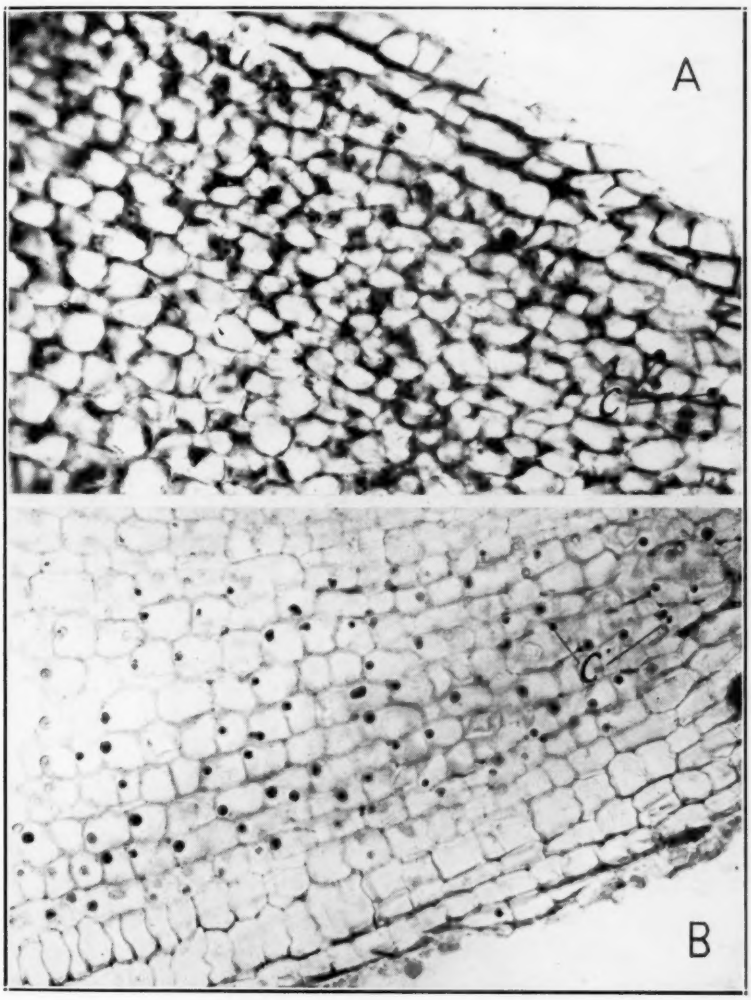

FIG. 2. A. Portion of a longisection of the post-meristematic region of a healthy orange root grown in a complete nutrient solution. The cell vacuoles were free of globular aggregates. B. Portion of a longisection of an orange root-tip that had grown in a zinc-deficient nutrient solution. In contrast to the condition shown in A, the vacuoles of many cells contained globular aggregates (stained with safranin). C, coacervates.

cortical parenchyma of an orange root tip that received all necessary nutrients. The rhomboidal cells shown in this figure contained many short, rod-like mitochondria, arranged along the cytoplasmic strand. The mitochondria had a pronounced tendency to form chains, together with an incipi-

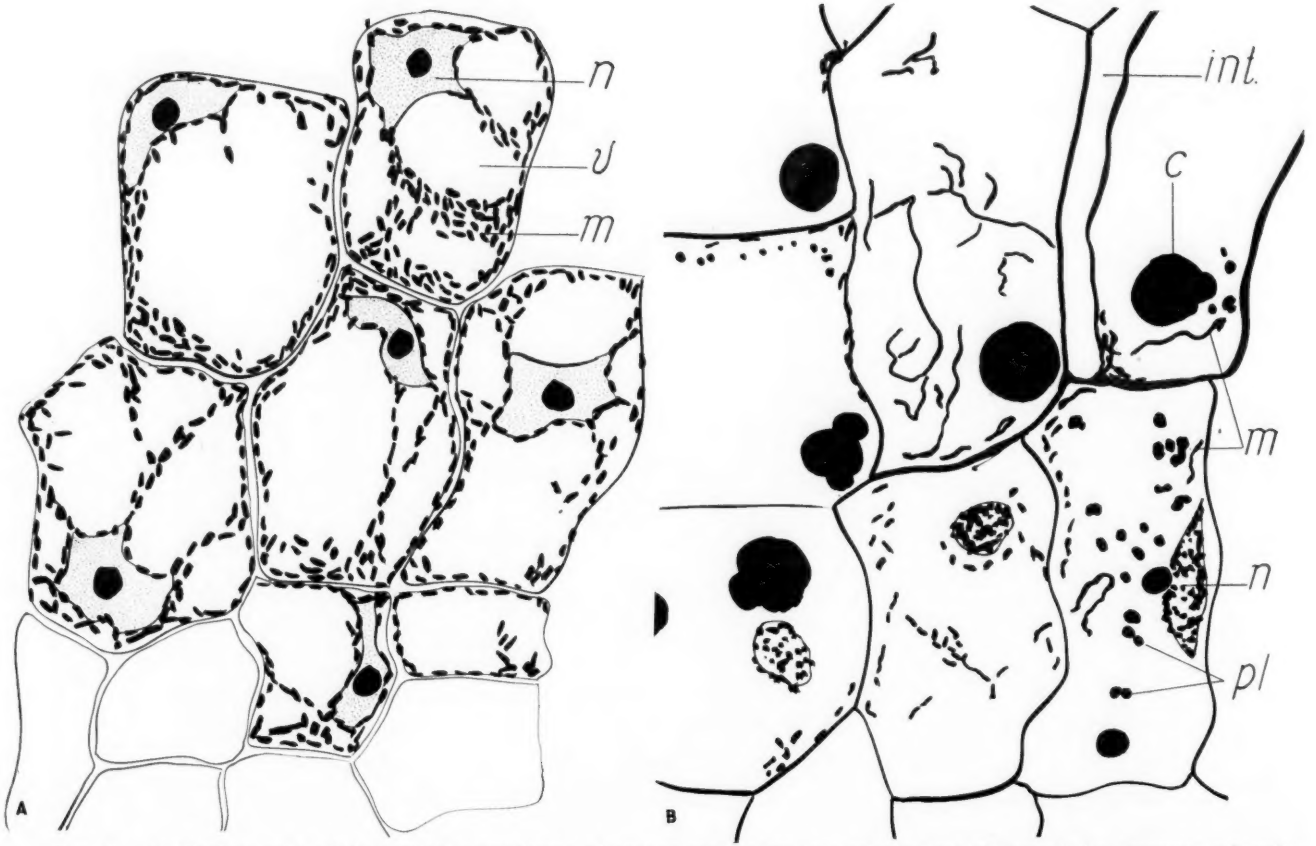

FIG. 3. A. Healthy cells of the cortical parenchyma of orange root grown in a solution containing all the necessary microelements. Vacuolar inclusions were not present. B. Hypoplastic cells from orange roots grown in a solution lacking zinc. In contrast to the healthy cells the vacuoles contained coacervated bodies, and skeinlike mitochondria. The coherence of mitochondria in some of the cells indicates a lowered surface tension. *n*, nucleus; *v*, vacuole; *m*, mitochondria; *pl*, plastids; *c*, coacervates; *int.* intercellular space.

ent development of amyloplasts, with one single starch grain. These amyloplasts were in definite contrast with the huge amyloplasts with numerous starch grains, found in cells of roots deficient in zinc. The nuclei of these cells were large and in many cases were deformed by the pressure of the vacuolar sap.

A striking contrast resulting from the deficiency of zinc in the nutrient solution is shown by cells of an orange root, seen in detail in figure 3, B. The large vacuoles contained conspicuous aggregates consisting of coacervated polyphenol contrasting with the optically empty vacuoles shown in figure 3, A. These coacervates had a tendency to cohere and to produce lobate bodies. It may be recalled that de Jong gave examples of lobate coacervates of casein formed at its isoelectric point, which were characterized by a very low water content.

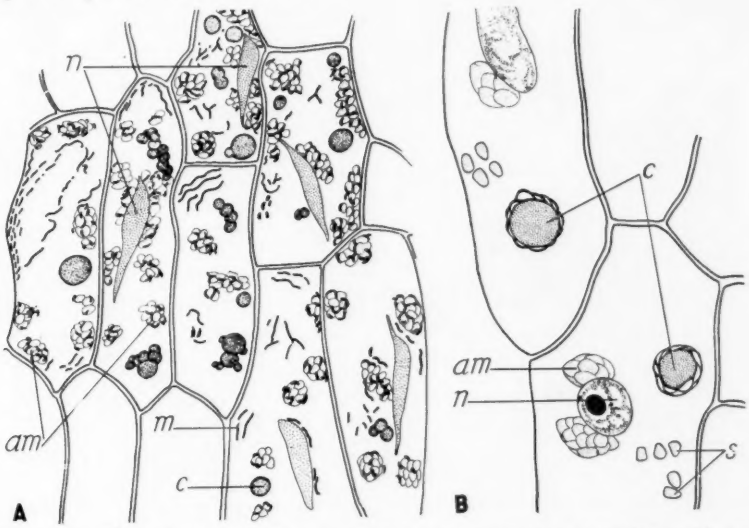

FIG. 4. A. Hypoplastic cells from an orange root grown in a solution lacking all the microelements. The cells were characterized by the large spindle-shape nuclei, by coacervated inclusions, large amyloplasts filled with starch grains, and by filiform mitochondria, stained with acid fuchsin and methyl green. B. Inclusions in cells of an orange root grown without zinc. *am*, amyloplast containing many starch grains; *c*, coacervates showing vacuolate envelope of phospholipoid; *n*, nucleus; *s*, starch.

Stated in its simplest terms it seems that we are observing colloidal bodies that have been separated from the liquid stage by "unmixing" similar to that described in a preceding paragraph. The importance of this simplified drawing will become evident as the discussion proceeds.

The effects of a deficiency of all the supplementary micro-elements on certain cyto-physiological relations of the cell are shown (Fig. 4, A) by cells of an orange root. Cells here represented came from an older part of the root than those in figure 3, B, and contained many typical coacervates. Here again it was evident that the smaller coacervates had a tendency to adhere to the larger coacervates forming characteristic clumps. It should be emphasized that coacervates cohere only with other coacervates and with

no other structures found in the cell vacuole. The presence of amyloplasts containing numerous starch grains indicates that the amylolytic agencies were inhibited in these hypoplastic cells; consequently, starch was not used in metabolism. The nuclei were abnormally large and were forced into spindle shapes by the mutual pressure of the cell vacuoles. The enlargement of the nuclei would in itself indicate that the cells were in an unhealthy condition. Coacervates, occurring singly and in clumps, could be seen in most of the postmeristematic cells. Especially conspicuous were the definite bounding membranes, owing to their strong affinity for acid fuchsin. The picture elucidates some of the salient features of the abnormal physiology of the cells suffering from mineral deficiencies and strongly emphasizes the fact now clearly established that under these conditions plastic building materials were not used for the maintenance of the plant, but accumulated rather in the form of conspicuous aggregates in the cell vacuoles.

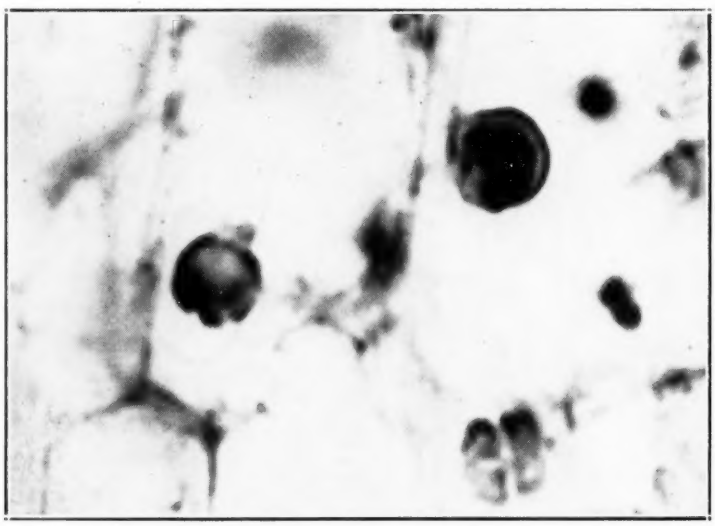

FIG. 5. Coacervated aggregates after treatment with molybdenic reagent, showing the alveolate nature of the surface layers.

Essentially the same cyto-physiological condition was found in other orange root-tips grown in solutions where all of the ordinary nutrient elements were furnished and the only missing microelement was zinc. Figure 4, B shows the large nuclei, the large coacervates with a strong tendency to clump together, and the amyloplasts surcharged with starch, indicating that something had so disarranged their equilibrium that the starch was not used for metabolism. By means of the molybdenum reagent we were able to demonstrate by the characteristic blue color that the delimiting membrane of the coacervate contains phosphoric material. The contrast between the coacervate and the cytoplasm and nucleus was beautifully shown in sections stained with safranin and methyl green, where the coacervates were stained dark-red and the cytoplasm and nuclei a bluish-green.

The nature of the lipoid envelope, which contains water as well as lipoid, may be inferred from the drawings in figure 4, B, and the photomicrograph shown in figure 5. These preparations were obtained by use of the molybdenic acid reagent, which imparts a blue color to compounds containing phosphorus. The amyloplasts also stained blue with this reagent, but more faintly, indicating that they are phosphorylated. In sections correctly handled with the molybdenum reagent, it was possible to see that the surrounding membrane of the coacervate was of an alveolate nature (Fig. 4, B), a fact not demonstrated by the use of such dyes as acid fuchsin. The molybdenum reagent also colored the central mass of the coacervate light-brown, due to the oxidation of the polyphenol to another substance, probably a diketone. In addition to the enveloping membrane, which

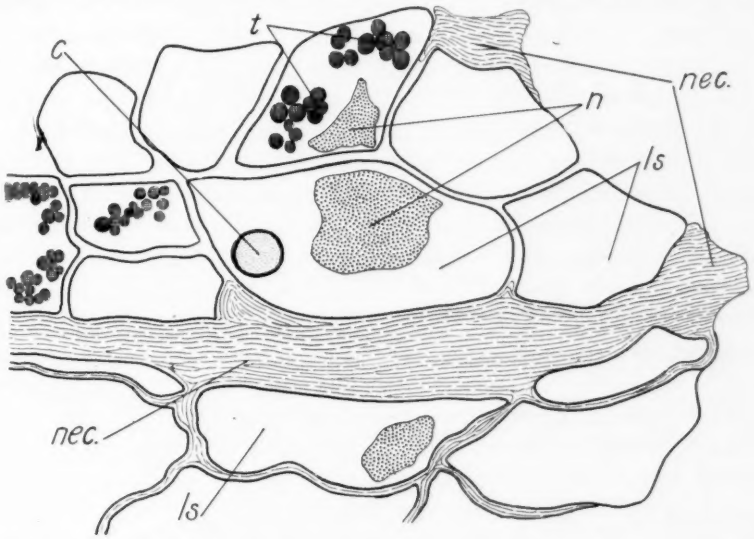

FIG. 6. Cells of a sunflower root showing types of disorganization resulting from boron and zinc deficiency. *c*, coacervate; *t*, globules of phenolic material; *ls*, example of lysis which destroyed the cytoplasm and its derivatives; *nec*, necrotic material; *n*, nucleus.

stained blue with the molybdenic reagent, the figures show that many of the coacervates tend to bulge at the surface. The same thing was seen in sections stained with acid fuchsin, a dye that is also differentially absorbed by phospholipoids.

An inspection of cells like those shown in figures 3, B, and 4, A, strongly suggests that the surface tension of mitochondria and plastids was greatly reduced in the affected cells. The straight or sinuous filaments of mitochondria formed by coherence were characteristic of hypoplastic cells. The adherence of mitochondria to nuclei and plastids illustrated in figure 4, A, is also an indication of the lowered surface tension of the solution. The writers (14) have previously described a characteristic polarization and clumping of the cell contents in affected leaves.

The disturbance in cell physiology caused by a deficiency of boron was

revealed by a study of cells in root-tips of sunflowers that had grown for 6 weeks in nutrient solution without any of the accessory substances present. The tops of the plants showed very evident injury from lack of boron and zinc.

Figures 3, B, and 6 show sections of root tips that contain large intercellular spaces, often filled with amorphous material that appeared to be a product of the necrosis of preexisting cell layers. The condition shown in figure 6 indicates that there had been extensive necrosis in the pericycle leaving a band of disintegrated material that absorbed haematoxylin very readily. The adjacent cortical cells that were still living contained globular coacervates or phenolic material. The extent of the injury resulting from the deficiency of boron and zinc is shown also by the lysis that affected the cytoplasm and its derivatives. Eltinge and Reed (4) found similar intercellular spaces in the roots of tomato plants suffering from zinc deficiency, and containing dark-staining amorphous material, which arose from disintegration of cellular material.

We conclude, therefore, from these converging lines of evidence, that we are dealing with a system in which complex coacervates are formed through a change in the equilibrium between the diffused colloidal material and the surrounding liquid. In this relation of molecular orientation the phosphatids probably play a significant role as factors of coacervation because the positively charged choline group is held at the surface of the polyphenol globule and the negatively charged phosphate ions, with adherent hydrocarbon chains, radiate outwardly into the dispersion medium (6).

The coacervated aggregates appear to consist of a central mass of phenols or polyphenols surrounded by a layer of phospho-lipoid material, formed as a result of the disturbance of hydrogen bonds in the catechol-water system by the activity of a catechol oxidase.

The evidence here presented, in addition to the discussion we previously published, Reed and Dufrenoy (12) points the way to a better interpretation of the conditions in the cells of diseased plants. It appears that important processes are intimately dependent upon small amounts of ions, such as zinc and boron, which tend to keep polyphenols and phospholipoids dispersed in the vacuoles of living cells. When they are absent, there may be a lowering of "solvation," resulting in flocculation or in coacervation. The specific activity of these metallic ions remains to be investigated.

SUMMARY

Our previous observations upon globular refringent bodies in the cells of abnormal leaves have been extended now to other parts of plants. These bodies have been found in roots and buds, as well as in leaves, and are interpreted as evidence that there has been a decided shift in the metabolic equilibrium of the cells.

Chemists have shown recently that colloids, which were dispersed in an

aqueous medium, may become concentrated into definite, stable globules by a process analogous to flocculation and have designated them as coacervates.

The coacervates herewith described in affected plant cells are considered colloids (mostly made up of catechol compounds) that separated out from the suspending fluid and were, consequently, condensed into spherical bodies floating in the saline solution of the vacuole. Those condensed colloids are surrounded by a precipitation membrane made up of phosphatids, meaning a complex of lipoids and phosphorus.

Those colloids, chiefly at the periphery, at the interphase with the limiting membrane, are the site for high oxidase activity. Therefore, they become the site for the attraction of positively charged ions and, conversely, a center of dispersion for negatively charged ions, such as PO_4. Phosphatids, however, may be considered to be amphoteric, and, therefore, could concentrate at the limiting surface, at the interphase, there to build up a limiting membrane.

Coacervates were found in the vacuoles of cells of roots grown in solutions lacking one or more of the important supplementary micro-elements. These bodies, however, were not found in the cells of roots receiving the necessary micro-elements. They were identified by their form, their stain reactions, and their location in the cells.

LITERATURE CITED

1. COLLANDER, R., und H. BÄRLUND. Permeabilitätstudien an *Chara ceratophylla*. Acta Bot. Fenn. **11**: 1–114. 1933.
2. COPLEY, M. J., G. F. ZELLHOEFER, and C. S. MARVEL. Hydrogen bonds involving the C–H link. IV. The effect of solvent association on solubility. Jour. Amer. Chem. Soc. **60**: 2666–2673. 1938.
3. ———, ———, and ———. Hydrogen bonds involving the C–H link. VIII. The solubilities of completely halogenated methanes in organic solvents. Jour. Amer. Chem. Soc. **61**: 3550–3552. 1938.
4. ELTINGE, E., and H. S. REED. The effect of zinc deficiency upon the root of *Lycopersicum esculentum*. Amer. Jour. Bot. **27**: 331–335. 1940.
5. HILDEBRAND, J. Order and disorder in pure liquids and solutions. Science **90**: 1–8. 1939.
6. JONG, H. G. Bungenberg de. La coacervation, les coacervates et leur importance en biologie. Hermann et Cie. (Paris). 1936.
7. ———, and H. R. KRUYT. Coacervation (Partial miscibility in colloid systems). Kon. Akademie van Wetensch. (Amsterdam) **32**: (2) 849–856. 1929.
8. KLERCKER, J. E. F. AF. Studien über die Gerbstoffvacuolen. Beih. K. Svenska Vet-Akad. Handlingar 13 (III) No. 8, pp. 63. 1888.
9. MCBAIN, J. W. and F. KELLOGG. The salting out of gelatin into two liquid layers with sodium chloride and other salts. Jour. Gen. Physiol. **12**: 1–15. 1928.
10. MASSEE, G. The "spot" disease of orchids. Ann. Bot. **9**: 421–429. 1895.
11. MOLISCH, H. Ueber amorphes und kristallisiertes Anthokyan. Bot. Zeit. **63**: 145–162. 1905.
12. REED, H. S., ET J. DUFRENOY. Effets de l'affection dite "mottle-leaf" sur la structure cellulaire des citrus. Rev. Gén. Botanique. **46**: 33–45. 1934.
13. ———, and ———. The effect of zinc and iron salts on the cell structure of mottled orange leaves. Hilgardia **9**: 113–137. 1935.
14. ———, and ———. Modification in cell structure accompanying mottle-leaf of the orange. Amer. Jour. Bot. **22**: 311–328. 1935.
15. RUSSELL, A. The natural tannins. Chem. Rev. **17**: 112. 1935.
16. ZELLHOEFER, G. F., M. J. COPLEY, and C. S. MARVEL. Halogen bonds involving the the C–H link. The solubility of halogens in donor solvents. Jour. Amer. Chem. Soc. **60**: 1337–1343. 1938.

THE TRANSIENT FEEDING OF ROOT-KNOT NEMATODE LARVAE[1]

M. B. Linford

(Accepted for publication August 6, 1941)

Difficulties inherent in the examination of living nematodes in association with their hosts have impeded investigations of how these parasites enter roots and how they induce the local necrosis, hypertrophy, or abnormal tissue differentiation characteristically associated with different root-infesting species. In 1937, the writer (5) described the feeding habits of the root-knot nematode, *Heterodera marioni* (Cornu) Goodey 1932, after it had attained its permanent site, and thereby helped to clarify the problem of how this parasite stimulates the development of giant cells and galls, but he did not determine when feeding begins nor how the nematode makes its way into a root. Certain pathological effects of this nematode are still not well defined, for they are induced before the larva has completed its penetration into and migration within its host to its site of permanent feeding. New refinements of techniques have now permitted observation of larvae before and during their entry into roots, with results that further elucidate the pathogenesis of root knot.

LITERATURE REVIEW

At the time Christie (2)[2] reported his thorough histological study of the development of root-knot nematode galls, the manner in which this parasite feeds was still the subject of hypotheses that Linford (5) later determined, by examination of living material, to be somewhat erroneous. It was found that giant cells develop under the stimulation of a persistently repeated cycle that includes: (a) puncture of the cell wall by the protrusible hollow buccal stylet or spear of the nematode; (b) injection of saliva into the cell; and (c) sucking out of only part of the cell content. Those observations were made on nematodes that had completed their entry into roots and had assumed their fixed positions for feeding. The few inadequate observations of larvae still in migration failed to detect feeding but did demonstrate that the stylet is used in opening a passageway.

The initiation of pathological disturbances prior to the parasite's reaching its permanent feeding site has been indicated by various investigators. Godfrey and Oliveira (3) found that heavy infestations may retard or stop root elongation within 24 hours and cause visible lateral enlargement of the root tip within 48 hours, even though the larvae have not, within that time, reached their feeding sites. They state (p. 329) ". . . the apparent swelling is due to retardation of forward growth of the root and abnormally

[1] Published with the approval of the director as Technical Paper No. 136 of the Pineapple Research Institute, University of Hawaii.

[2] See this paper for a comprehensive statement of the development of galls and of the group of highly modified giant cells at the head of the parasite, with a review of earlier literature.

directed growth of the weakened meristematic tissues rather than to true gall development."

Christie (2) observed that when considerable numbers of larvae enter a root simultaneously, growth may be stopped within 24 hours, and suggested that the presence of the parasite in the meristem suppresses mitotic activity. He also found very prompt reaction of root cells to the presence of the larvae, showing first in hypertrophy of cortical cells ". . . not confined to cells lying adjacent to the path of the larva . . . ," and also sometimes of endodermal and pericyclic cells. Some cells are destroyed and some passed through by the migrating larva, Christie found, constituting exceptions to the general rule that migration is intracellular with little injury to the cells.

Linford (9), studying the development of cowpea shoots from experimentally infested buds, found severe leaf deformities, sometimes out of proportion to numbers of nematodes present, as well as stoppage of terminal buds; and suggested that these disturbances might be results of mechanical injuries inflicted by migration of larvae through meristematic tissues, separating the cells and thereby disturbing the coordination of tissue differentiation.

Godfrey and Oliveira (3) and also Linford (7) have called attention to the phenomenon of mass attack upon root tips, numerous larvae entering the root in closely packed groups, and Linford (9) has demonstrated experimentally the tendency for larvae to enter through wounds where such are available, but these workers have not considered the factor of mechanical injury resulting from such mass attacks in the etiology of nematode galls.

METHODS

Observation of the activities of larvae before and during penetration into roots required the development of a technique that would permit observation at high magnification with both roots and nematodes firmly supported in a moist medium but with the larvae free to move. Details of apparatus and methods are reported elsewhere (10). Briefly, however, roots were allowed to grow against a coverslip in soil or other granular medium, either in a ring chamber formed by cementing a coverslip onto a glass ring, or in a miniature root observation box (8) modified by cementing a coverslip over an opening in one side. For very small seedlings the ring chamber with a thin layer of dark soil or sand was excellent if the chambers were held in a moist chamber between observations. Observation was with the aid of a 40× achromatic water immersion objective and cooled incident light. Somewhat of a dark-field effect was obtained, revealing ample detail to permit identification of the nematode species and observation of its activities. Where the host protoplasm was not too abundant it sometimes was possible to see significant details through 1 or even 2 layers of root cells.

Some of the observations of active migration and of host injuries were made with washed roots and transmitted light. Use also was made of materials processed with either Fleming's fluid and clove oil or lactophenol with

cotton (anilin) blue or acid fuchsin. Fixation for paraffin sections was in Karpechenko's fluid.

Heterodera marioni larvae for these studies were hatched from egg masses on cowpea roots, but were of a strain obtained originally from a pineapple field. After hatching in shallow water they were decanted and finally passed through filter paper as described elsewhere (9) to free them from débris.

Observations were made with roots of various plants, although lettuce was employed somewhat more than others. Primary roots of *Portulaca grandiflora* have the advantage of being so slender and translucent that activities of nematodes may be observed dimly almost in the center of the root by either reflected or transmitted light. Such slender roots are less satisfactory for observation of the early stages of penetration because their small radius of curvature leaves only a narrow surface in contact with the glass, and relatively few nematodes penetrate from a position advantageous for observation.

OBSERVATIONS

The Process of Feeding

The feeding process in young, transient *Heterodera marioni* larvae is very similar to that already described (5) for older individuals. The larva punctures a cell with its stylet, then lies at rest briefly before sucking out part of the cell contents.

The puncturing of a cell wall is accomplished only with difficulty despite the extreme slenderness of the stylet tip. A larva thrusts its stylet chiefly when its body is so braced that it may hold its head firmly against a resistant surface; but, when it is favorably situated and is apparently trying to puncture a cell wall, the rapidity and persistency with which it thrusts are remarkable. One larva, observed continuously during 36 minutes, was seen to thrust its stylet at approximately the following rates, expressed as numbers per 30 seconds: 52, while the larva slowly moved its head over two cells; 81, with the head held securely in one place; 58, while again exploring; and 107, immediately before puncturing a cell. By thrusting at such rates and sometimes for many minutes against a small area of a single cell, the nematode gradually weakens the resistance of the wall. Perhaps diffusion of some substance through the injured wall may provide the stimulus for the nematode to persist in its attack in one place and finally to exert the special effort required for puncturing. After a usually prolonged series of thrusts in one place with gradually quickening tempo, the stylet is finally inserted into the cell with a series of three or more very rapid jerks, the stylet not sliding back between thrusts, and each one carrying it farther forward until the maximum possible degree of protrusion is achieved.

Once the stylet tip is fully inserted into a cell, all perceptible motion of the nematode stops. Sometimes, after only a few seconds, the stylet is withdrawn either with or without 2 or 3 preliminary pulsations of the esophageal bulb, and the nematode moves to another cell. Usually, however, after a

period of 15 to 30 seconds of rest, the esophageal bulb begins a rapid and rhythmic pulsation that may continue 10 to 40 seconds. In the young larva this bulb is relatively inconspicuous and the valve at its center is so small that the action of the bulb can be recognized only with good definition at high magnification. Conditions of observation amply sufficient to detect activity of the bulb in a half-grown or mature female are wholly inadequate here. Pulsation is very rapid, as indicated by the following approximate numbers of complete pulsations per 30 seconds: 90, 95, 120, 91, 95, 93 and 104. These rates were computed from counts made during the usually shorter periods of pulsation while one larva fed on the surface of a lettuce root. It has not been possible in this study to observe movement of cell contents toward the

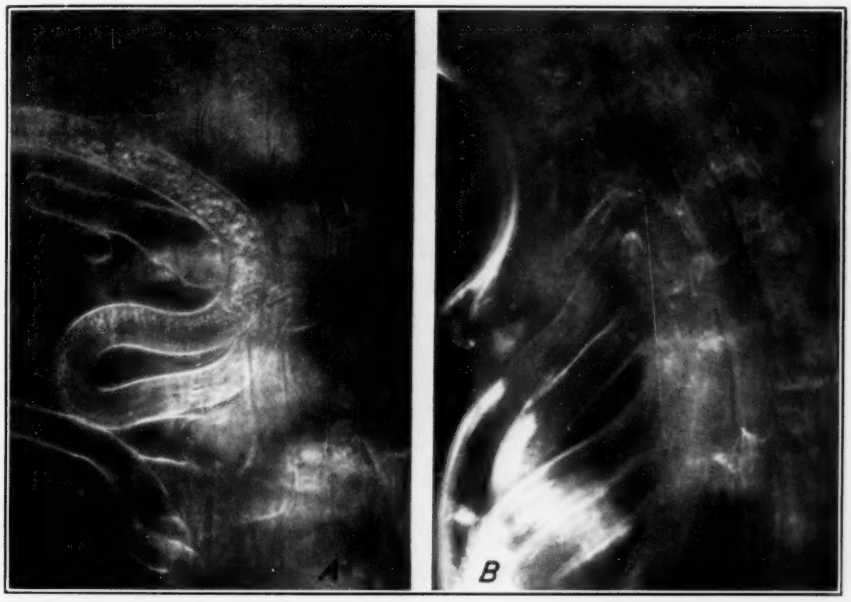

FIG. 1. *Heterodera marioni* larvae associated with lettuce roots, photographed by incident light. ×380. A. Larva in position for initial attempt to feed. B. Group of larvae attacking at a point where injury, begun earlier, has already caused the root to curve.

stylet tip nor into the anterior end of the intestine, but evidence from other studies (6) leaves no doubt that this rhythmic pulsation of the esophageal bulb is the mechanism with which such nematodes injest their food.

As soon as action of the bulb stops, the larva retracts its stylet and either immediately resumes thrusting in approximately the same place, turns its head to a different cell, or crawls to a different position before the next period of feeding.

Feeding from Epidermal Cells

A larva may begin thrusting its stylet intermittently soon after it makes contact with the root, even in a relatively mature zone (Fig. 1, A), but successful piercing of a wall has not been seen in epidermal tissues older than

where root hairs are beginning to develop. As has been described previously (7), the larva characteristically migrates down the root toward its tip. Some feeding occurs in the old to intermediate elongating zone, but a larva rarely persists in such a place long enough to enter the root. Instead, after feeding in a cell or two, it advances to the young elongating zone or the meristematic region. During such movement it may feed in several cells, and in one cell several times. No instance of feeding in a root hair itself was observed, although several times larvae were seen to feed in the basal portion of a cell from which a root hair was developing. Some epidermal cells look no different under the conditions of observation employed here after having been fed in than do normal cells, but dermatogen cells, after having been fed in several times, frequently appear much disorganized. Time intervals between separate feedings are varied, even when the larva is in a favorable site and is beginning to penetrate, commonly ranging from two to four minutes. Much longer intervals may occur when feeding is interrupted by locomotion.

Feeding During Entry and Migration Within the Root

After a dermatogen or young epidermal cell has been fed in one or more times and battered by innumerable stylet thrusts during a period of minutes, its wall may break, allowing the head of the nematode to slip in while the disorganized remains of the protoplast flow out into the water film on the root surface. The larva then commonly continues to penetrate into the root, feeding as it goes. Bacteria and protozoa congregate at such points and may enter the wound. Other larvae also may be attracted to this point (Fig. 1, B), where they attempt to invade the root together. With the still heavier infestations illustrated by Godfrey and Oliveira (3) and by Linford (7), the larvae crowd so closely together that directional movement of the individual is limited. Within such masses they may lie almost motionless but continue thrusting their stylets and breaking down numerous cells. Apparently, they disturb one another to such an extent that they less frequently are able to feed than when distributed singly, and certainly larvae in groups are less favorable for observation of the details of penetration.

After an individual has forced its head into a cell, its feeding continues much as on the root surface, periods of rapid thrusting of the stylet alternating with periods of rest and of sucking. It uses its stylet also both to break through walls and to separate cells along the middle lamella. A larva may break into subepidermal cells—masses of larvae characteristically do—but generally, as reported by other investigators, the individual larva pushes between cells as it moves farther into the root. Sometimes, where a subepidermal cell has been entered, it has been possible to observe the thrusting of the stylet into the second layer of cortical cells, with activities similar to those on the root surface. Even when the head of the nematode could no longer be seen, the occasional period of pulsation of the esophageal bulb gave evidence that the same mechanism of feeding was persisting.

A few fortunate observations have been made on larvae that were migrat-

ing in a subepidermal position, crowding between cells of the first subepidermal layer, so that feeding and migration could still be observed after $\frac{2}{3}$ of the body length was within the root. Such larvae fed repeatedly from cells on either side of the head, and also thrust the stylet directly between cells. These larvae were in young tissues where cell walls appeared to afford little resistance, and the intervals between feedings were shorter and more regular than on the root surface or in a more mature part of the root. As on the root surface, one cell frequently was fed in more than once. These larvae, in agreement with numerous others that have been observed after penetration was almost complete, entered the root with a halting motion: even when

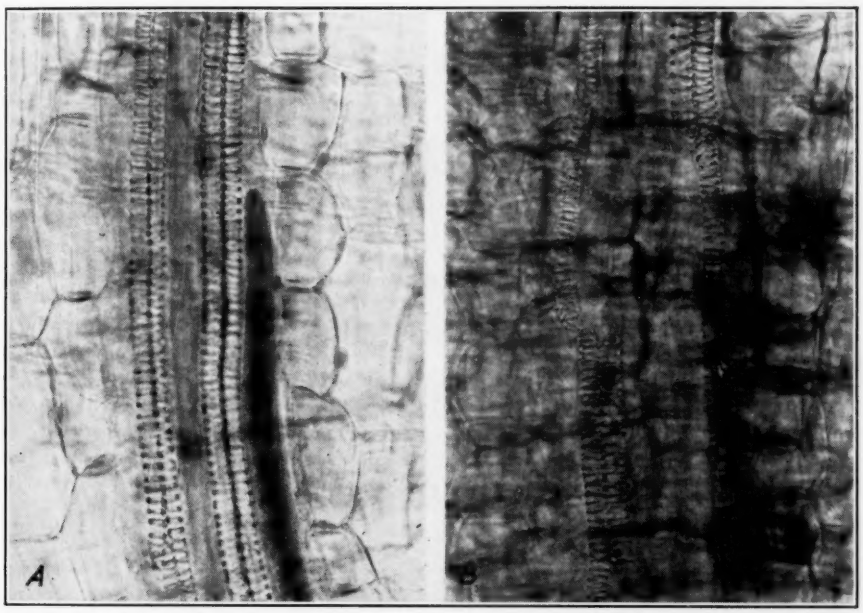

FIG. 2. *Portulaca grandiflora* root processed with lactophenol and anilin blue 2 days after infestation with *Heterodera marioni* larvae. The root tip was distinctly swollen, but the only 3 larvae present were in the mature stele, migrating up the root. ×380. A. Larva between endodermis and xylem, apparently wedged between pericycle cells. Two other larvae, not shown, were between the primary xylem bundles. B. Part of the swollen apical region, showing wide separation of xylem bundles and interrupted xylem elements. The dark areas at right of center were brown, apparently marking the path of entry of one or more larvae.

only a little of the tail was exposed to view, the nematode was seen to advance approximately one cell length or less, and then lie at rest for 2 minutes or more before moving farther in. Such halting advance has been observed with larvae penetrating almost directly into the meristem through the side of the root cap and with others entering the maturing zone. Consequently, there is reason to think that intermittent feeding characteristically continues throughout the period of migration. Exceptions have been noted, such as larvae observed within the stele of *Portulaca grandiflora* roots where they were completely surrounded by well differentiated tissues (Fig. 2, A). Such

larvae, observed in the living condition before the root was processed, appeared to be trapped in spaces so narrow that they were unable to turn about, and between walls too heavy for them either to break down or puncture for feeding.

Entering larvae very commonly are found headed toward the root tip, perhaps in consequence both of having migrated down along the root surface

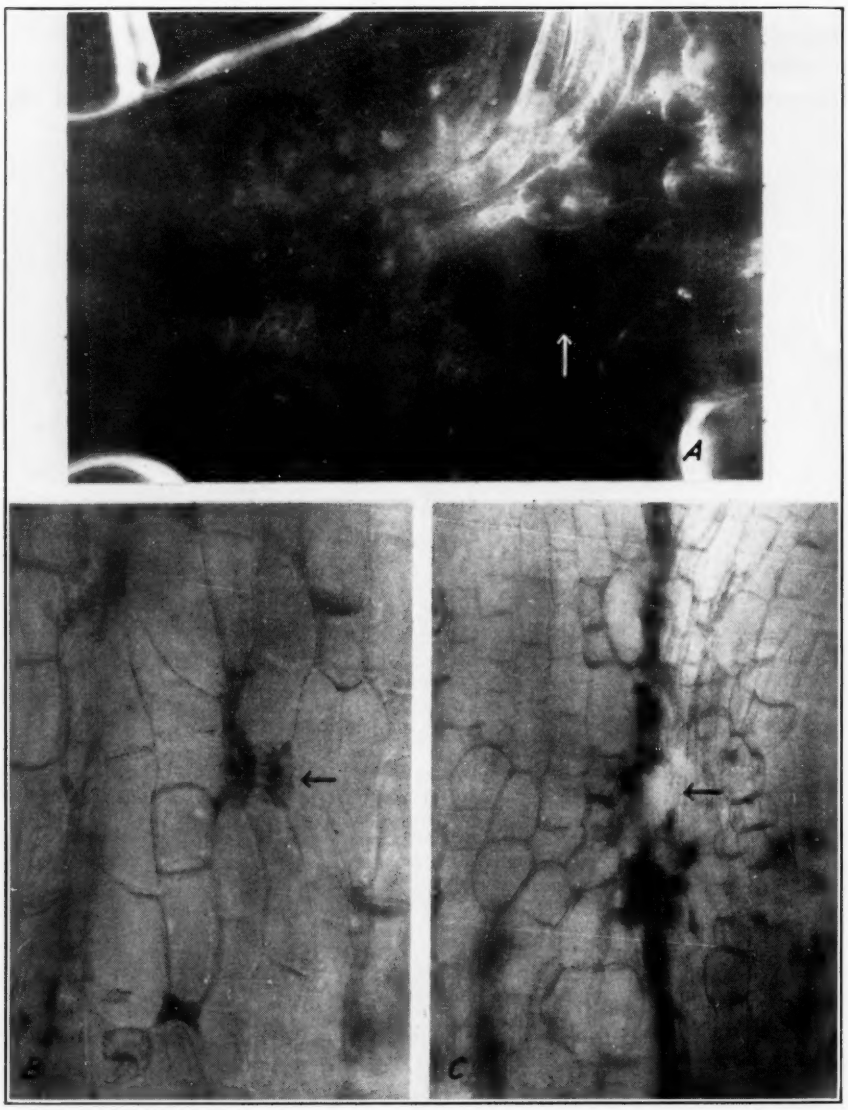

FIG. 3. A. Several larvae (upper right) entering a Portulaca root at the edge of a deep wound (at arrow) begun by earlier penetrants and enlarged by growth of less injured tissues. Photographed by incident light. ×380. B. Disturbed cell pattern in young epidermis of lettuce root infested 50 hours earlier with *H. marioni* larvae. The arrow marks a hole left by incomplete closure of a destroyed cell. ×380. C. Like B, but with a conspicuous line of injury and with a deep hole at arrow. ×190.

prior to penetration, and of the forward motion of the root tip through the surrounding medium during early stages of entry. Many such larvae, in slender roots, continue in the same general direction to the terminal meristem where the altered orientation of cells or contact with less nutritious root cap cells cause them to turn about and start back up the root.

Some Pathological Effects

As reported by Christie (2) and earlier workers, larvae move chiefly between cells without destroying them. In paraffin sections, however, the writer finds a tendency for cells just behind the head of a migrating nematode to show compact nuclei and sometimes a mild shrinkage of the entire cell. These changes may represent abnormal responses to the fixing fluid, but probably the basic cause is the altered cell constitution resulting from feeding. No attempt has been made in this study to follow cytological changes in these cells, but several slender roots have been seen showing hypertrophy where larvae have not established for permanent feeding. The *Portulaca grandiflora* root shown in figure 2, A, B, contained 3 larvae, all in the stele of the mature zone. The root tip was conspicuously swollen, and the stele itself was hypertrophied, evidently in consequence of earlier feeding of these larvae during migration through the meristem.

Cells destroyed by the entering larvae are much more numerous in the dermatogen and outer periblem than farther in. Distributed singly or in small groups, such broken cells tend to be crushed closed by enlargement of adjacent tissues, leaving only minor irregularities in root shape beyond the disturbed cell pattern (Fig. 3, B, C). More extensive injuries, resulting from the attack of numerous larvae on a small root, sometimes result in deep holes (Fig. 3, A) or in cracks that may extend into the differentiating stele. Such cracking probably is attributable in part to hypertrophy such as Christie (2) found to begin promptly after larvae enter a root, but here occurring more abundantly and under a broken epidermis.

In the most extreme instances, the entire root tip is disintegrated without gall formation. This has been observed with roots of various plants attacked by numbers of larvae comparable to those shown in figure 1 of a preceding paper (7). In none of these observations have bacteria been excluded, but from the promptness of disintegration when well washed larvae were added to essentially aseptic seedling cultures, as well as from the actual observation of tissue destruction by larvae, there is little doubt that the nematodes themselves are basically the cause of such disintegration of root tips.

DISCUSSION

The rhythmic processes of feeding shown by young *Heterodera marioni* larvae on the root surface and during entry into the root are essentially similar to those described earlier for large females, the chief differences being in rates. Not only does the larva rest more briefly after inserting its stylet into a cell, and then feed for a shorter time, but also its stylet thrusts and the

pulsation of its bulb are more rapid. No evidence was obtained that the young larva injects saliva into the cell before sucking out cell contents, but the period of rest prior to pulsation appears analogous with the periods of demonstrated saliva flow in predacious species of *Aphelenchoides* (6) and in *Heterodera marioni* females (5). The essentially non-lethal effects of the feeding of these larvae is attested by the scarcity of dead cells along paths of migration. The hypertrophy of some such cells described by Christie (2), the false gall formation reported by Godfrey and Oliveira (3), and the distortion of some lightly infested aerial parts reported by Linford (9) apparently are all related to the feeding of migrating larvae.

The factors that cause a larva to terminate its migration present an interesting question. Mere selection of cells in which it is able to feed does not answer, otherwise larvae would not have left the meristematic region and migrated back into the mature zone, as shown in figure 2, A, B. This condition has been seen only in very slender roots, suggesting that migration stops only after the larva has already fed during some moderate period. These observations also raise, but do not answer, the question of whether a larva too nearly starved to be able to enter a root may, by feeding on the root surface, so replenish its reserves that it can later expend the energy required to force entry.

Such physical injuries to root tips as are here described probably are negligible at the beginning of the crop season under normal conditions. After hatching of first or second generation eggs has begun, however, especially from exposed egg masses, local concentrations of larvae undoubtedly become sufficient to terminate growth of new rootlets in close proximity to such egg masses. Many instances of rootlet disintegration formerly regarded by the writer as results of mildly pathogenic microorganisms invading roots of plants weakened by root knot, now appear primarily attributable to the root-knot nematode itself.

The observed entry of microorganisms from the soil into superficial root cells destroyed by the penetrating larva raises the question of why bacteria are not conspicuously associated with the enlarging or mature nematode at its permanent feeding site. Ark and Thomas (1) and Kalinenko (4) have demonstrated that bacteria commonly accompany certain nematodes that, however, enter chiefly into maturing tissues rather than near the apical meristem. Perhaps both the gradual posterior taper of the *Heterodera marioni* larva to a relatively slender terminus, and the vitality of meristematic and enlarging root cells, result in too prompt and complete closure of the migration path for bacteria to be carried far in appreciable numbers. Or possibly bacteria do play a rôle in gall formation that has not yet been recognized.

The ability of *Heterodera* larvae to both macerate root tips and cause cracks that extend into a differentiating stele may be as significant as the early disintegration of galls in the observed tendency for heavy root-knot infestations to accentuate Fusarium wilt of cotton, even in wilt-resistant varieties.

SUMMARY

The feeding of *Heterodera marioni* larvae was observed at 600 diameters magnification with cool, incident light and with roots growing in soil or other granular medium against coverslips.

Larvae may feed repeatedly from epidermal cells before entering the root. They continue feeding as they enter into and migrate within it.

The stylet tip is inserted into a cell after rapid and persistent thrusting against the wall; the larva then lies apparently at rest 15 to 30 seconds (analogous with the period of saliva flow), next sucks 10 to 40 seconds with its esophageal bulb pulsating vigorously, and finally retracts its stylet.

Young epidermal or dermatogen cells, especially at the point of entry of the nematode, are killed not by feeding but by entry of the larva after the wall has been weakened by the stylet. Individual larvae similarly kill some cells within the root.

Larvae attacking in groups destroy many cells, causing holes, cracks into the young stele, or, with extremely heavy infestations, disintegration of root tips.

Feeding during entry and migration appears to explain cessation of elongation and the swelling of root tips while larvae are migrating, as well as the early hypertrophy of cells remote from the permanent feeding site.

LITERATURE CITED

1. ARK, P. A., and H. EARL THOMAS. *Anguillulina pratensis* in relation to root injury of apple and other fruit trees. Phytopath. **26**: 1128–1134. 1936.
2. CHRISTIE, J. R. The development of root-knot nematode galls. Phytopath. **26**: 1–22. 1936.
3. GODFREY, G. H., and JULIETTE OLIVEIRA. The development of the root-knot nematode in relation to root tissues of pineapple and cowpea. Phytopath. 22: 325–348. 1932.
4. KALINENKO, V. O. The inoculation of phytopathogenic microbes into rubber-bearing plants by nematodes. Phytopath. Zeitschrift **9**: 407–416. 1936.
5. LINFORD, M. B. The feeding of the root-knot nematode in root tissue and nutrient solution. Phytopath. **27**: 824–835. 1937.
6. ————. The feeding of some hollow-stylet nematodes. Proc. Helminth. Soc. Wash. **4**: 41–47. 1937.
7. ————. Attractiveness of roots and excised shoot tissues to certain nematodes. Proc. Helminth. Soc. Wash. **6**: 11–18. 1939.
8. ————. A miniature root-observation box. Phytopath. **30**: 348–349. 1940.
9. ————. Parasitism of the root-knot nematode in leaves and stems. Phytopath. **31**: 634–648. 1941.
10. ————. Methods of observing soil flora and fauna associated with roots. Soil Science **53**: 93–103. 1942.

STUDIES ON BLACK STEM OF ALFALFA CAUSED BY ASCOCHYTA IMPERFECTA[1]

MAURICE L. PETERSON AND L. E. MELCHERS[2, 3]

(Accepted for publication October 23, 1941)

INTRODUCTION

A black-stem disease of alfalfa in Kansas, the cause of which had not previously been published, has been observed for many years. It occasionally produces serious defoliation and death of the stems during cool wet spring seasons.

Although black stem is of less economic importance in the dry climate of Kansas, seed from this State often is used in humid regions where this disease is more prevalent. This paper deals with a study of the characteristics of the disease, the identification of the causal organism and the methods used in artificial inoculations suitable for a systematic testing of alfalfa breeding material. Development of black-stem resistant selections of alfalfa offers one method of control.

REVIEW OF LITERATURE

Stewart, French, and Wilson (8) of New York described black stem of alfalfa in 1908, and attributed it to an undescribed species of *Ascochyta*. The fungus was named *Ascochyta imperfecta* in 1911 by C. H. Peck (3).

Valleau and Fergus (10) in 1929 reported a similar disease of unknown origin on alfalfa, sweet clover, and red clover, in Kentucky, which they referred to as black stem. In 1933, Johnson and Valleau (2) found a different organism on each of these legumes. The disease of alfalfa was attributed to *Phoma medicaginis* Malbr. and Roum. A black-stem disease of alfalfa was reported in Idaho in 1936 by Remsberg and Hungerford (4), which was considered the same as that reported by Valleau and Fergus, and Johnson and Valleau, in Kentucky. Toovey, Waterston, and Brooks (9) of England compared cultures of *Phoma medicaginis* Malbr. and Roum., received from F. R. Jones of Wisconsin and E. M. Johnson of Kentucky, and *Ascochyta imperfecta* Peck, received from Jones and R. Sprague, with the British fungus causing black stem, and concluded that all of these cultures were the same species, namely, *Ascochyta imperfecta* Peck. In 1934, Richards (5) of Utah reported a stem blight on alfalfa of undetermined origin which closely resembled the disease caused by *A. imperfecta*. Similar diseases of alfalfa are attributed to *Ascochyta medicaginis* Fckl. by Rosella (6) in France, and *Phyllosticta medicaginis* (Fckl.) Sacc. by Corneli (1) in Italy.

[1] Contribution from the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, cooperating with the Department of Botany, contribution No. 420 and the Department of Agronomy, contribution No. 327, Kansas Agricultural Experiment Station, Manhattan, Kansas.

[2] Junior Agronomist, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Head, Department of Botany, Kansas Agricultural Experiment Station, respectively.

[3] The authors gratefully acknowledge the assistance and advice of Dr. D. B. Creager, C. O. Grandfield, and C. O. Johnston during the course of these studies.

There is a lack of agreement on the cause of black stem of alfalfa, and some of the conclusions reached in the literature are indefinite. In view of this fact the writers believed it essential to determine the cause of this disease as it occurs in Kansas, before attempts were made to study other phases of the problem.

THE DISEASE

Economic Importance and Geographic Distribution

Detailed observations of black stem were made in the alfalfa plots of the Kansas Agricultural Experiment Station at Manhattan, in 1939. Although infection was light compared with that of previous seasons, a loss of over 15 per cent of the leaves occurred on some of the plots. This loss is of importance when it is considered that the leaves contain a much higher percentage of protein and minerals than the stems. In seasons of severe infection many shoots are entirely killed.

Johnson and Valleau (2) reported that during long, wet spring seasons black-stem kills the early crop of shoots, forcing new crown buds to push out from below the soil surface. On poor soil during favorable black-stem seasons infection was severe enough to destroy stands. Richards (5) reported a loss of 40 to 50 per cent of the yield during a severe outbreak in Utah.

The disease has been found in many parts of Kansas and Nebraska. It also has been reported in New York (8), Kentucky (2, 10), Utah (5), and Idaho (4). In England (9) it was reported in Hertfordshire, Suffolk, Norfolk, and Bedfordshire and it may have been this same disease which was reported in France (6) and Italy (1).

SIGNS AND SYMPTOMS

The earliest symptoms in spring are small, dark-brown or black spots on the stems and leaves. Young stem lesions often are surrounded by water-soaked areas that become slightly raised in later stages of growth. Severely infected stems turn dark brown or black and die (Fig. 1, A). Immature pycnidia develop in the older stem lesions in the winter. By the following spring the stems are covered with numerous black pycnidia (Fig. 1, D).

Under cool humid conditions, the leaf lesions enlarge rapidly, coalesce, and vary in size and shape. They are occasionally zoned and surrounded by chlorotic areas. Petiole lesions occur as numerous, small, black or brown spots, or elongated, diseased areas, which partly or entirely girdle the petiole causing the leaflet to wither. Stipules may become diseased (Fig. 1, C). Diseased leaves turn yellow and wither before they drop. Such leaves usually contain obscure, immature pycnidia. Leaf shedding commences at the bottom of the stem and progresses upwards as the disease becomes more severe (Fig. 1, B).

Field Observations

The extent of the disease in a field is directly correlated with temperature and humidity. It is most severe on the first crop of alfalfa, which usually

is produced during the coolest and wettest period of the growing season. In Kansas the infection is negligible on the second and third cuttings, but increases considerably on the fourth crop, when the weather is again cooler

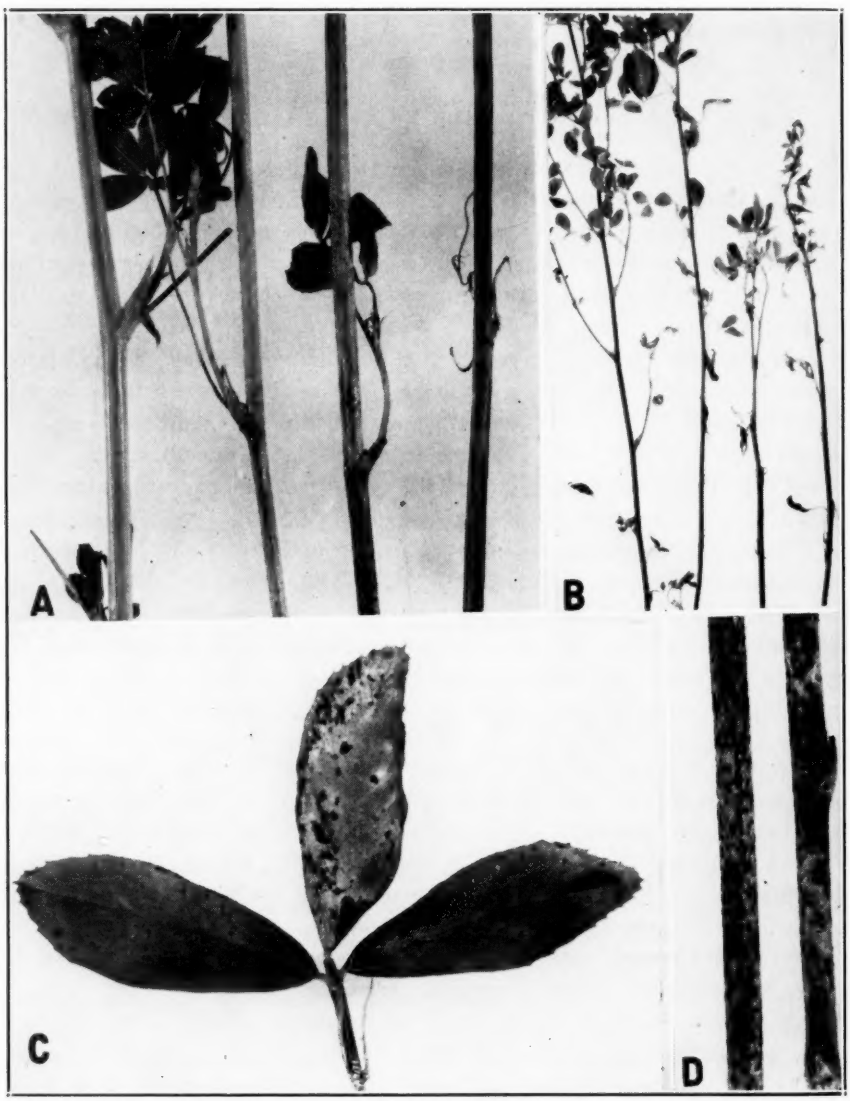

FIG. 1. A. Progressive stages (left to right) in the development of black stem on alfalfa stems. B. Progressive stages of defoliation (left to right) of alfalfa resulting from increased severity of infection by black-stem; C. Black-stem lesions on the petiole, stipules, and leaves of alfalfa; D. Pycnidia of *Ascochyta imperfecta* on overwintered stems of alfalfa.

and more humid. Small lesions may be observed on leaves and stems in 3 or 4 days following a rain. Prolonged cloudy, rainy weather is favorable for numerous infection periods and rapid spread and development of the disease.

THE CAUSAL ORGANISM

The causal organism of black-stem of alfalfa in Kansas was identified as *Ascochyta imperfecta* Peck. On potato-dextrose agar, the mycelium was greyish, olive-green, with a whitish margin, and produced abundant pycnidia containing both uniseptate and unicellular spores of variable size, but of dimensions within the range of 6–15 × 2.5–4 μ as first described by Peck (3) and later by Toovey, *et al.* (9).

Life History

Infection takes place early in the spring when the first shoots of alfalfa appear. Spores are disseminated by rain drops that strike diseased overwintered leaves and stems (Fig. 1, D). The spores germinate within a few hours and the germ tubes enter the alfalfa leaves and stems, probably by direct penetration. The mycelium grows rapidly through the tissue, and diseased areas may be seen with the naked eye 3 to 4 days after infection has taken place. Infected tissue becomes necrotic and pycnidia soon develop.

The organism remains dormant in the host tissue during the dry, hot season, but fruiting is resumed in the fall, with the coming of cool, humid conditions. The inoculum for the fourth cutting was found originating chiefly from diseased crop residue from the first cutting. Spores are expelled in a continuous stream through the ostiole of moistened pycnidia, following rains. Diseased crop residue from the fourth cutting is the most prolific source of inoculum for spring infection, although spores remain viable in diseased stems and leaves from other cuttings. Maturation of pycnidia takes place in early winter, when temperature and moisture conditions are favorable, but probably both the mycelium and pycnidia remain dormant during the winter months.

Temperature Relations

Temperature relations of *Ascochyta imperfecta* were determined by measuring the mycelial growth on plates of potato-dextrose agar at 3-degree intervals, using a range from 9° to 33° C.

Spores originating from single hyphal-tip cultures were allowed to germinate on the agar before the plates were transferred to the incubators. Growth measurements were made 4 days after exposing 2 series of 45 plates each to 9 different temperatures.

Growth was very slow at 9°, but increased up to 21° and fell off slightly at 24°. Above 24° growth decreased rapidly; very little occurred at 33° (Fig. 2).

The margins of colonies were of a frayed and cottony appearance in the 9° to 18° temperatures, but became clearly defined at the higher temperatures. The cottony margins of the colonies where no fruiting occurred became narrower with increasing temperatures. Pycnidia were produced at the periphery of the colonies at 27° and 30°, but none occurred at 33°. Fruiting took place from 9° to 30°. Pycnidia, produced at lower temperatures, were

small and translucent but became darker at higher temperatures. The cultures kept at the 18° to 24° temperatures were olive green. The 27° cultures were darker colored, while the 30° and 33° cultures were dark- and light-tan, respectively.

Further observations of the effect of temperature on fruiting were made on sterile alfalfa leaves. Leaves of uniform size and age were selected from a single plant and placed on microscope slides. These were placed in small moist chambers. Three leaves were placed on each of 27 slides in a like number of moist chambers and autoclaved at 15 lb. for 20 min. After cool-

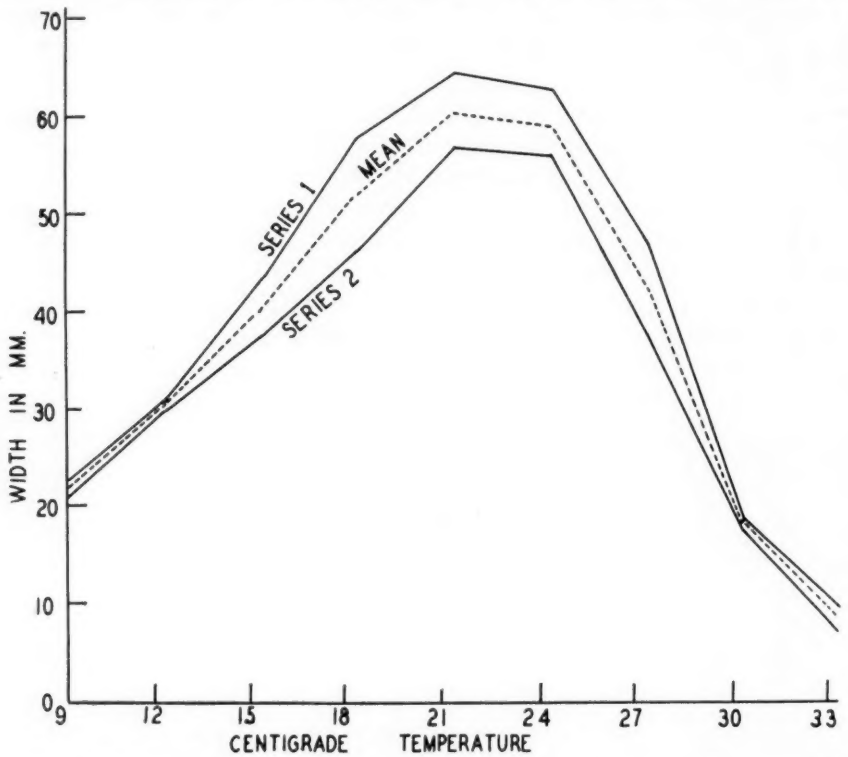

FIG. 2. Temperature relations of *Ascochyta imperfecta* determined by measuring the width of colony at the end of four days.

ing, a suspension of spores from a pure culture was placed on each leaf, and 36 hours were allowed for germination before placing the leaves in the incubators. After 6 days the leaves were carefully examined.

Pycnidia were produced throughout the range of temperatures, but were exceptionally abundant at 21° to 27°. They were larger at 21° and 24° than at any other temperature, but most numerous at 27°. Approximately 1,500 pycnidia were counted on one leaf at the 27° temperature. These pycnidia produced no spores during the experiment.

Inoculation Studies

The fungus was grown on potato-dextrose agar, alfalfa stems, alfalfa

leaves, and sweet-clover stems in an effort to determine the most satisfactory medium for the production of a large supply of viable spores for artificial inoculations. The best medium proved to be sterile, second-year sweet-clover stems. They were kept moist with an inch of water agar in the bottom of the test tube. Sweet-clover stems inoculated with spores along one side developed cottony mycelium, with pycnidia appearing later (Fig. 3). Spores from cultures prepared in this manner were viable after storage in a refrigerator at about 40° F. for 6 months.

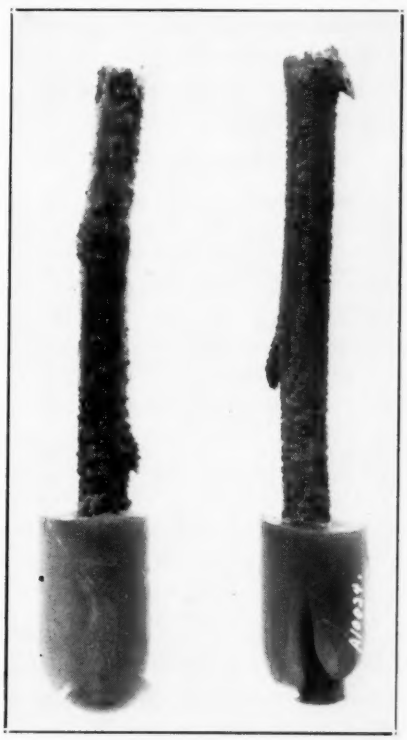

FIG. 3. Pycnidia of *Ascochyta imperfecta* on sterile sweet clover stems.

Inoculations on alfalfa were made by spraying spore suspensions in distilled water on healthy plants that were then placed in a moist chamber for 3 to 5 days, depending upon the season of the year and greenhouse temperatures. A shorter time was required in winter. The relative humidity in the damp chamber was between 90 and 100 per cent, and the temperature between 65° and 70° F., except in summer, when it was higher. Typical young black-stem lesions developed on the leaves, petioles, and stems of alfalfa within 3 days. Stem lesions, however, were less abundant than in the field. On several occasions the fungus was reisolated from inoculated plants and compared with the inoculum, thereby proving its identity and pathogenicity.

The effect of age of the leaf upon ease of infection by the black-stem

organism was determined by charting the growth of 3 plants each of Turkestan and Ladak varieties that had previously been cut back to the ground. The purpose of conducting this experiment was to determine if the recovery of different plants or varieties following cutting was a factor affecting susceptibility. The plants were artificially inoculated after charting growth for 23 days. When sufficient time had elapsed for infection, each leaf was scored from 1 to 5, depending upon severity of infection, using as a guide a photograph of diseased leaves illustrating the severity of disease on leaves representative of each number. Consideration was given to size and abundance of lesions. The disease score of leaves of an age group was determined by averaging the score of all leaves within the group. The results are shown in table 1. There is no relationship between leaf age and severity of infection, as determined by this experiment.

TABLE 1

Numerical disease score of leaves of various ages on six alfalfa plants

Plant No.	Age of leaves in days									
	0–4		5–9		10–14		15–19		20–23	
	No. of leaves	Disease	No. of leaves	Disease	No. of leaves	Disease	No. of leaves	Disease	No. of leaves	Disease
Turk. 1	7	2.14	7	2.29	12	2.00	11	2.00	9	2.11
" 2	12	5.00	16	4.81	16	4.69	22	5.00	2	5.00
" 3	16	4.69	13	4.69	18	4.61	24	4.88	0	.....
Ladak 1	9	3.88	8	4.13	12	3.08	11	2.82	2	2.50
" 2	9	3.22	9	2.88	12	2.42	15	4.33	0	.....
" 3	10	2.70	10	2.30	12	2.50	8	3.13	0	.....
Av.	10.5	3.61	10.5	3.52	13.7	3.22	15.2	3.69	4.3	3.20

HOST RANGE

Toovey, Waterston, and Brooks (9) secured slight spotting of leaves on *Vicia sativa* L. and lesions similar to those on alfalfa on *Trifolium pratense* L., and *Medicago lupulina* L. by artificial inoculation with *Ascochyta imperfecta*. Sprague (7) secured infection to a slight degree on *Melilotus indica* (L.) All., *M. officinalis* (L.) Lam., *Trifolium hybridum* L., and *T. pratense* L. Johnson and Valleau (2) report that *Phoma medicaginis* produced blackening of the stems of red clover and sweet clover, as well as those of alfalfa. No other studies seem to have been made on this phase of the work.

The investigation herein reported served to test the susceptibility of varieties and selections in the field and greenhouse. In connection with the variety test, approximately 50 plants each of *Medicago falcata* L. and *M. ruthenica* Trautv. were found to produce typical lesions when artificially inoculated with *Ascochyta imperfecta*. These species, however, were more resistant to black stem than was common alfalfa. Certain varieties and plants within a variety showed definite indications of resistance. In some cases resistance appeared to be associated with glossy leaves that possibly shed moisture.

Studies on varietal resistance, and other methods of control of alfalfa black stem will be reported in a forthcoming paper.

SUMMARY

Black stem of alfalfa, occurring in Kansas, has been traced to *Ascochyta imperfecta* Peck. This disease causes destructive defoliation and discoloration of the hay crop. It is most severe during cool moist weather, and the first crop of hay is more heavily infected than subsequent cuttings.

The organism probably overwinters as dormant mycelium and pycnidia in crop residue. Field infection takes place by splattering raindrops, which carry spores from diseased tissue to the growing alfalfa shoots.

The optimum temperature for growth of the pathogen in culture was 21° C. Slight growth was observed at 9°, and very little occurred at 33°. Pycnidia were produced throughout the range from 9° to 30°, and were most abundant at 27°.

The most satisfactory method of producing viable spores for artificial inoculations was to grow the fungus on sterile sweet-clover stems in test tubes. Inoculations were made by spraying spore suspensions on healthy plants and placing them in moist chambers from 3 to 5 days. No relationship existed between age of alfalfa leaves and severity of infection following artificial inoculation.

Medicago falcata L. and *M. ruthenica* Trautv. were added to the host range reported in literature.

LITERATURE CITED

1. CORNELI, E. Sopra una grave alterazione della Medica prodatta da 'Phyllostieta medicaginis' Fuck. a Perugia. Rev. Path. Veg. **22**: 3–4, 51–58. 1932.
2. JOHNSON, E. M., and W. D. VALLEAU. Black-stem of alfalfa, red clover, and sweet clover. Kentucky Agr. Exp. Stat. Bull. 339. 82 p. 1933.
3. PECK, C. H. Report of the State Botanist, 1911. Species not before reported. New York State Mus. Bull. 157. 21 p. 1912.
4. REMSBERG, RUTH, and C. W. HUNGERFORD. Black-stem of alfalfa in Idaho. Phytopath. **26**: 1015–1020. 1936.
5. RICHARDS, B. L. Reaction of alfalfa varieties to stem blight. Phytopath. **24**: 824–827. 1934.
6. ROSELLA, E. Observations sur L'*Ascochyta* de la Luzerne. Rev. Path. Vég. **16**: 226–229. 1929.
7. SPRAGUE, R. Host range and life-history studies of some leguminous Ascochytae. Phytopath. **19**: 917–932. 1929.
8. STEWART, F. C., G. T. FRENCH, and J. K. WILSON. Troubles of alfalfa in New York. New York (Geneva) Agr. Exp. Stat. Bull. 305. 416 p. 1908.
9. TOOVEY, F. W., J. M. WATERSTON, and F. T. BROOKS. Observations on the black-stem disease of lucerne in Britain. Ann. of App. Biol. **23**: 705–717. 1936.
10. VALLEAU, W. D., and E. N. FERGUS. Black-stem of alfalfa, sweet clover, and red clover. Phytopath. **19**: 507–509. 1929.

LIPOLYTIC ACTIVITY OF PHYTOPATHOGENIC BACTERIA DETERMINED BY MEANS OF SPIRIT BLUE AGAR AND ITS TAXONOMIC SIGNIFICANCE

MORTIMER P. STARR[1] AND WALTER H. BURKHOLDER[2, 3]

(Accepted for publication October 23, 1941)

The action of phytopathogenic bacteria on fats has received scant attention from students of this group of microorganisms. Jodidi (14) demonstrated crystals of the higher fatty acids in a skim-milk culture of *Bacterium pruni* and accepted this as evidence that this species produces lipolytic enzymes. Castell (5), by means of a modification of Turner's (18) Nile-blue medium, and Castell and Garrard (6), by means of a titrametric technique, showed that *Phytomonas campestris* could decompose fats and that *Erwinia carotovora* lacked this ability. No other mention of lipolytic activity in cultures of related plant pathogens has been found despite a thorough search of the available literature.

Probably one of the reasons why the ability to decompose fats has been overlooked, in studying the physiology of these bacteria, is that methods for detection of this character had not been sufficiently developed to be readily applicable to routine use. It seemed possible that the spirit-blue-agar technique, recently developed by the senior writer (17), might prove satisfactory for this purpose. This medium is essentially a tryptone-yeast extract agar containing a finely dispersed cottonseed-oil emulsion and the indicator dye, spirit blue. It is prepared in sterilized Petri plates for streaking. The fresh sterile medium is pale lavender, if the pH be properly adjusted. Lipolytic[4] bacteria, streaked on the agar, cause the development of a *permanent*, characteristic, deep-blue color beneath and around the colony; colonies of non-lipolytic[4] bacteria produce no comparable change.

It is the writers' purpose to report here on the decomposition of cottonseed oil by various phytopathogenic bacteria as shown by the spirit-blue-agar method, and to evaluate this ability as a taxonomic character.

EXPERIMENTAL

Cultures of the organisms listed in the tables were streaked on plates of spirit-blue cottonseed-oil agar (17). Control plates lacking the cottonseed oil usually were inoculated at the same time, with the intention of guarding against the possibility of development of the blue reaction in the absence of

[1] Brooklyn College, Brooklyn, New York.
[2] Cornell University, Ithaca, New York.

[3] The writers wish to express their appreciation to those workers, who by their generous contributions of cultures, have furthered this investigation. An extension of this work is in progress and receipt of any authentic cultures of plant pathogenic bacteria will be welcomed.

[4] The actual reaction shown by spirit-blue agar is possibly more than hydrolysis of the fat. In this paper, the writers wish the term *lipolysis* to indicate that particular action on cottonseed oil that is revealed by the spirit-blue-agar technique, without committing themselves as to the actual mechanism of that action.

fat. No control plate of this type ever showed a spurious reaction. As an additional precaution, each new batch of the medium was tested with a series of bacteria of known fat-decomposing ability. All cultures were incubated at 27–30° C. and observed periodically for not less than two weeks. Each culture was studied in this manner at least twice and each of the reactions given in the tables is thus the resultant of numerous independent observations.

In the following tables, the species of the genus *Phytomonas* Bergey *et al.* are allocated to the genera or groups that have been suggested (1, 2, 3, 10, 13) as indicating more correctly the true relationships of these organisms; the reasons for accepting these groupings are discussed more fully below. This division should occasion little inconvenience, since it is essentially the arrangement of the species in the fifth edition of Bergey's *Manual* (2).

DISCUSSION

Examination of these data reveals that the members of the genus *Phytomonas* Bergey *et al.* vary in their ability to decompose cottonseed oil, as shown by the spirit-blue-agar method. This might be expected, since the genus was erected mainly on the basis of plant pathogenicity and, actually, as various investigators (1, 3, 9, 10, 13) have shown, consists of several physiologically unlike groups. In the discussions that follow, it will be shown that the members of any one of these groups usually have a similar and characteristic reaction on spirit-blue agar.

The possible value of lipolysis as a taxonomic character may be indicated by the fact that, regardless of source or age of culture, isolates of any one species give identical reactions by the spirit-blue-agar method. For example, none of the 11 isolates of *Pseudomonas syringae*, received from different laboratories under 9 synonyms, were lipolytic by this method. A similar situation obtained with *Xanthomonas phaseoli*, in which case all the isolates, including two varieties, were lipolytic, even though some of the strains were more than 15 years old. Additional confirmation will be found in the tables.

Consideration of the data in the tables may be more revealing if the fat-splitting ability of each of the diverse groups which comprise the genus *Phytomonas* Bergey *et al.* is discussed in turn.

Xanthomonas. The yellow-pigmented, monotrichous or nonmotile, slime-forming, gram-negative, nonsporing, rod-shaped bacteria, which may cause disease in plants, were placed by Dowson (10) in the new genus, *Xanthomonas*. This genus, with certain exceptions, is synonymous with the subgroup, "Phytomonas proper," of the genus *Phytomonas* Bergey *et al.* (2), and with those species frequently referred to as the *Phytomonas campestris* group. For reasons advanced and reviewed by Dowson (10) and supported by the junior writer (3, 4), it seems desirable to refer to these organisms as *Xanthomonas* species, especially since Elliott (11) has pointed out that *Phytomonas* is a homonym.

The *Xanthomonas* species are actively lipolytic by the spirit-blue cottonseed-oil agar method. The data included in table 1, show that isolates of

TABLE 1.—*The lipolytic activity of some strains of Xanthomonas on spirit-blue cottonseed-oil agar*

Species	Source	Lipolytic ability
Xanthomonas barbareae	WHB[a] BI, BR	Active
Xanthomonas begoniae	Oregon WHB 96	Active
" "	WHB	Active
Xanthomonas campestris	WHB 85, 86, R4	Active
" "	Oregon WHB C 22	Active
Xanthomonas campestris v. *armoraciae*	WHB HI	Active
Xanthomonas corylina	Oregon 5401, 5402, 5403	Active
" "	WHB 78	Active
Xanthomonas geranii	WHB G1, G2, G3, 87, 89	Active
Xanthomonas gummisudans	WHB 94	Active
Xanthomonas hederae	WHB 16	None
Xanthomonas holcicola	Elliott 102	Active
Xanthomonas juglandis	WHB 77	Active
" "	WHB 79	Slight
" "	Oregon 5080, 5171, 5184	Active
Xanthomonas malvacearum	Braun B87	None
" "	MPS[a] 1, 2, 3, 4	None
Xanthomonas papavericola	WHB 47, 48	Active
Xanthomonas pelargonii	WHB 100, WHB 92	Active
" "	Oregon WHB 93	Active
Xanthomonas phaseoli	WHB E2	Active
" "	Oregon WHB 5	Active
" "	Braun B16	Active
" "	Hedges	Active
" "	MPS 1941	Active
Xanthomonas phaseoli v. *fuscans*	WHB 8	Active
Xanthomonas phaseoli v. *sojense*	WHB 11	Active
Xanthomonas pruni	Oregon WHB 24	Active
" "	Braun B65, B67	Active
" "	Thornberry 1, 2, 3	Active
Xanthomonas rubrilineans	Braun B90	None
Xanthomonas translucens	WHB 61, 90	Active
Xanthomonas translucens v. *undulosa*	WHB 83, 91	Active
Xanthomonas vasculorum	WHB 49	Active
Xanthomonas vesicatoria	Pepper WHB 1, 2, 3, 4, 5, 6, 7, 99	Active
" "	Tomato WHB 13, 16, 38, VI	Active
" "	Unknown Ark	Active
Xanthomonas vesicatoria v. *raphani*	Lincoln	Active
Xanthomonas sp. (Kendrick's stock, *Matthiola incana*, pathogen)	WHB St1	Questionable
" "	WHB St2, St3	Active

[a] The designation WHB indicates the culture collection of the junior writer; MPS, that of the senior writer.

21 of the 24 authentic species and varieties of *Xanthomonas* examined were able to decompose cottonseed oil. It should be noted that the previously mentioned observations of Jodidi (14) and of Castell (5, 6) concerning the lipolytic activity, respectively, of *X. pruni* and *X. campestris*, are thus confirmed. It is significant that *X. rubrilineans*, one of the few species in this

group that is not lipolytic, does not conform closely to certain other cultural characteristics of this genus.

Phytopathogenic Pseudomonas. The close relationship of the green-fluorescent, pigment-producing phytopathogenic bacteria now in the genus *Phytomonas* Bergey *et al.* to the saprophytes and animal pathogens now in Bergey's *Manual* in the genus *Pseudomonas* Migula has been shown by several workers. Rahn (15) has already pointed out the "practical impossi-

TABLE 2.—*The lipolytic activity of some phytopathogenic Pseudomonas on spirit-blue cottonseed-oil agar*

Species	Source	Lipolytic ability
Pseudomonas aceris	Ark	None
Pseudomonas alliicola	WHB[a] On1, AN, 1, 2, 4, R1, R2, R4	Active
Pseudomonas andropogoni	Braun B82	None
Pseudomonas angulata	Braun B99	None
Pseudomonas berberidis	WHB G35	None
Pseudomonas caryophylli	WHB RC, 3, 4, B, R2, R3, R8, RR7, RB, Car 1	Active
" "	WHB C, R4, Jones	Slight
Pseudomonas cichorii (*endiviae*)[b]	WHB G18	None
Pseudomonas coronafaciens	Elliott 75	None
Pseudomonas delphinii	WHB G36	None
Pseudomonas glycinea	WHB G8	None
Pseudomonas lachrymans	WHB G12	None
Pseudomonas lapsa	Ark	None
Pseudomonas marginata	WHB G40	Active
Pseudomonas maculicola	WHB G37, CI	None
Pseudomonas medicaginis	WHB G10	None
Pseudomonas medicaginis v. *phascolicola*	WHB 1, G3, G29, G31, A, MPS 1941	None
Pseudomonas mors-prunorum	Braun B69	None
Pseudomonas pisi	WHB G32	None
Pseudomonas polycolor	WHB G11	Active
Pseudomonas primulae	Ark	None
Pseudomonas striafaciens	Elliott 70	None
Pseudomonas syringae	WHB G7, SyF, Syl	None
Pseudomonas syringae (*vignae*)[b]	WHB G15	None
Pseudomonas syringae (*vignae* v. *leguminophila*)	Braun B45	None
Pseudomonas syringae (*cerasi* v. *prunicola*)	Braun B38	None
Pseudomonas syringae (*prunicola*)	Braun B70	None
Pseudomonas syringae (*holci*)	WHB G28	None
Pseudomonas syringae (*trifoliorum*)	WHB G20	None
Pseudomonas syringae (*cerasi*)	WHB G21	None
Pseudomonas syringae (*utiformica*)	WHB G24	None
Pseudomonas tabaci	Penn. State	None
Pseudomonas viburni	Thornberry 1, 2, 3	None
Pseudomonas viridiflava	WHB, G4, G5	None
Pseudomonas viridilivida	WHB G23	None
Pseudomonas woodsii	WHB G34	None

[a] See footnote, table 1.

[b] Species names in parentheses are those synonyms under which the cultures were received.

bilities" involved in a separation of these two groups of microorganisms based solely on the character of plant pathogenicity. The comparative studies of Clara (7), Seleen (16) and others have demonstrated the cultural similarity of these groups, and a merger of the two genera has been recommended (1, 3, 10). In this study, the green-fluorescent, and certain related but nonpigmented, plant pathogenic bacteria will be referred to as species of *Pseudomonas*.

The strong lipolytic activity of a number of *saprophytic* species of *Pseudomonas* has been reported (5, 6, 8). The writers, also, have observed that 15 isolates of the saprophyte, *Ps. fluorescens*, were strongly lipolytic by the spirit-blue cottonseed-oil-agar technique. Accordingly, it may be surprising to learn from the data in table 2 that the majority of *phytopathogenic Pseudomonas* species studied did not split cottonseed oil by this method. Of the 27 species tested, isolates only of 4 species were lipolytic. Of these lipolytic plant pathogenic species, at least one, *Ps. polycolor*, is culturally so closely related to the lipolytic saprophyte, *Ps. fluorescens*, that any attempt at a separation, except on the basis of plant pathogenicity, is at present impossible. The other 3 lipolytic species, *Ps. alliicola*, *Ps. caryophylli*, and *Ps. marginata*, have never been known to produce a green-fluorescent, diffusable pigment, and, possibly, are not now classified correctly.

The Gall-forming, Rhizobium-like Group. The junior writer (3) pointed out the close relationship of certain of the gall-forming phytopathogenic bacteria to the Rhizobiaceae Conn and, later, Conn, Wolfe, and Ford (9) and Hofer (12) discussed the taxonomy of these species. The data in table 3 indicate that the twelve strains of tumefying *Phytomonas*, cultured on spirit-blue cottonseed-oil agar, showed no typical lipolytic reaction,

TABLE 3.—*The lipolytic activity of the gall-forming phytopathogenic bacteria and of some related Rhizobiaceae on spirit-blue cottonseed-oil agar*

Species	Source	Lipolytic ability
Phytomonas gypsophilae	N. Brown	None
Phytomonas pseudotsugae	Hansen	None
Phytomonas rhizogenes	Hildebrand	None
Phytomonas savastanoi	Braun B40	None
" "	C. O. Smith	None
Phytomonas savastanoi v. *fraxini*	N. Brown	None
Phytomonas tonelliana	C. O. Smith	None
Phytomonas tumefaciens	A.T.C. 6408	None
" "	Hildebrand B2, BP	None
" "	Braun B2, B6	None
Rhizobium leguminosarum (*Medicago*)	MPS[a]	None
Rhizobium leguminosarum (*Arachis*)	MPS	None
Chromobacterium violaceum	MPS	None
Chromobacterium ianthinum	MPS	None
Alcaligenes fecalis	MPS	None
Alcaligenes radiobacter	Hofer	None

[a] See footnote, table 1.

although certain cultures caused a transient bluing of the medium, the significance of which is not yet understood. The fact that 6 strains belonging to the 3 genera of the Rhizobiaceae were also without demonstrable fat-splitting ability may be a further indication of the postulated relationship.

The Corynebacterium (Gram-positive) and Phytomonas stewartii (Gram-negative) Groups. On the basis of his extensive studies, Jensen (13) considered that at least 4 species of plant-pathogenic bacteria belong in the genus *Corynebacterium* Lehmann and Neumann. It is likely that other gram-positive species included in Bergey's *Manual* (2) as part of "Appendix II" of the present genus *Phytomonas* are also species of *Corynebacterium;* these are so indicated in table 4. Since the taxonomic position of

TABLE 4.—*The lipolytic activity of the phytopathogenic corynebacteria (gram-positive) and of the Phytomonas stewartii (gram-negative) group on spirit-blue cotton-seed-oil agar*

Species	Source	Lipolytic ability
Corynebacterium fascians	Oregon P1, P2	Slight or questionable
" "	Hildebrand R, S	Slight
Corynebacterium flaccumfaciens	WHB[a] S1, S2, S3, S4, S5, S6, S7, S9, S20, S31	None
Corynebacterium insidiosum	Oregon WHB S24	None
" "	Fitzpatrick A11, A21, A34, A39, A41, A42, A50, A54, A55, A57, A74, A77, A80, A81, A89, A91, A97, A99	None
Corynebacterium michiganensis	WHB S11, S18, S29	None
Phytomonas stewartii	Braun B93, B95	None
" "	Elliott 403517, 403558	None
" "	Lindstrom—virulent 3152, 3153, 3155, 3156	None
" "	Lindstrom—avirulent 3151, 3154	None
" "	WHB S13, S15, S16	None
Phytomonas manihotis	WHB 1, 2, 4, R1, R2, R3, R4	Slight or questionable
Phytomonas tardicrescens	WHB S26	None

[a] See footnote, table 1.

the gram-negative species, *Phytomonas stewartii, P. tardicrescens,* and *P. manihotis,* is rather uncertain, it will not be discussed further here, and the name *Phytomonas* is retained.

As shown in table 4, none of the phytopathogenic corynebacteria or of the gram-negative *Phytomonas stewartii* group are actively lipolytic by the spirit-blue-agar method. However, it should be noted that *Corynebacterium fascians* and *Phytomonas manihotis* show a weak or questionable reaction.

SUMMARY

The spirit-blue-agar technique was used for studying decomposition of cottonseed oil by phytopathogenic bacteria. Two hundred and six cultures belonging to 65 species and varieties of the genus *Phytomonas* Bergey *et al.* were examined by this method. The reaction on spirit-blue cottonseed-oil agar may be of value in classifying the plant-pathogenic bacteria, since iso-

lates of any one species give similar reactions regardless of source or age of the culture. Moreover, each of the groups comprising the genus *Phytomonas* Bergey *et al.* appears to have a tolerably characteristic ability to decompose fat as shown by this technique. Of 24 *Xanthomonas* species and varieties tested, 21 are actively lipolytic. Isolates of only 4 of the 27 species of phytopathogenic *Pseudomonas* are lipolytic; further study may reveal other, more correct, relationships for these lipolytic species. None of the 18 isolates of gall-forming plant pathogens and related Rhizobiaceae thus examined gave a typical lipolytic reaction. None of the tested species of phytopathogenic *Corynebacterium* or of the *Phytomonas stewartii* group are actively lipolytic, although in two species there was recorded a slow or questionable reaction. The taxonomy and nomenclature of the plant-pathogenic bacteria are discussed particularly in the light of this study.

LITERATURE CITED

1. BURKHOLDER, W. H. The genus *Phytomonas*. Phytopath. **20**: 1–23. 1930.
2. ———. *Phytomonas* Bergey *et al.* *In* Bergey's *Manual of Determinative Bacteriology*. pp. 142–218. Fifth edition. (Baltimore). 1939.
3. ———. The taxonomy and nomenclature of the phytopathogenic bacteria. Phytopath. **29**: 128–136. 1939.
4. ———. The black rot of *Barbarea vulgaris*. Phytopath. **31**: 347–348. 1941.
5. CASTELL, C. H. *p*-Aminodimethylaniline monohydrochloride as an indicator of microbial action on fats. Stain Techn. **16**: 33–36. 1941.
6. ———, and E. H. GARRARD. The action of microorganisms on fat. III. Oxidation and hydrolysis of triolein by pure cultures of bacteria. Canadian Jour. Res. *C*. **19**: 106–110. 1941.
7. CLARA, F. M. A comparative study of the green-fluorescent bacterial plant pathogens. Cornell Univ. Agr. Exp. Stat. Mem. **159**: 1–36. 1934.
8. COLLINS, M. A., and B. W. HAMMER. Types of lipolysis brought about by bacteria as shown by Nile-blue sulphate. Jour. Bact. **27**: 487–496. 1934.
9. CONN, H. J., G. E. WOLFE, and M. FORD. Taxonomic relationships of *Alcaligenes* spp. to certain soil saprophytes and plant parasites. Jour. Bact. **39**: 207–226. 1940.
10. DOWSON, W. J. On the systematic position and generic names of the gram-negative bacterial plant pathogens. Zentralbl. Bakt. u.s.w. Abt. II. **100**: 177–193. 1938.
11. ELLIOTT, C. The genus *Phytomonas*. Phytopath. **27**: 1181–1182. 1937.
12. HOFER, A. W. A characterization of *Bacterium radiobacter* (Beijerinck and van Delden) Löhnis. Jour. Bact. **41**: 193–224. 1941.
13. JENSEN, H. L. Studies on saprophytic mycobacteria and corynebacteria. Linn. Soc. N. S. W. Proc. **59**: 19–61. 1934.
14. JODIDI, S. L. The production of certain enzymes by *Bacterium pruni*. Jour. Agr. Res. [U. S.] **35**: 219–221. 1927.
15. RAHN, O. Contributions to the classification of bacteria. I. Practical impossibilities in taxonomy. Zentralbl. Bakt. u.s.w. Abt. II. **78**: 1–3. 1929.
16. SELEEN, W. A. A study of green-fluorescent pigment-producing organisms. Thesis. Cornell University. 1941.
17. STARR, M. P. Spirit blue agar: a medium for the detection of lipolytic microorganisms. Science **93**: 333–334. 1941.
18. TURNER, R. H. The action of bacteria on fat. I. Relative merits of various differential plating mediums for lipase-producing organisms. Jour. Inf. Dis. **44**: 126–133. 1929.

THE BROAD RING-SPOT VIRUS

JAMES JOHNSON AND ROBERT W. FULTON[1]

(Accepted for publication November 22, 1941)

Because of the number of viruses occurring naturally on tobacco (*Nicotiana tabacum* L.), and the common use of this host as a test plant for many others, it may be of some value to describe more fully a new virus reported from this laboratory in 1938 (2). This virus, to which the common name broad ring-spot was applied, is also of some interest because of its natural occurrence in only a single instance on tobacco, a circumstance that may eventually have some bearing on theories concerning the origin of viruses. Not only did the virus fail to appear in any other fields surveyed in 1938, but it has not been found since 1938 in the same or other fields. In 25 years of fairly extensive surveys for virus diseases in Wisconsin tobacco fields, it is not recalled that similar symptoms have ever been noted, nor have they been seen in numerous tobacco fields of other States inspected by the senior writer. The virus, therefore, should be regarded as extremely rare on tobacco. Nothing is known of its natural occurrence on other host plants, many of which are now known to be susceptible to the virus by artificial inoculation.

Broad ring spot was found in a 6-acre field of tobacco near Edgerton, Wisconsin, on a crop nearing the topping stage. About 30 per cent of the plants in the field showed distinct symptoms. The disease was at first thought to be ordinary tobacco ring spot (5), known not to occur naturally in Wisconsin. Specimens of the leaves were taken to the laboratory and photographed (Fig. 1, *A*). Young tobacco and tomato plants, in the greenhouse, were inoculated with extracts. Good infection and symptoms were readily obtained on both hosts (Fig. 1, *B* and *C*). Since ordinary tobacco ring-spot virus is not known to infect tomato by the extract method of inoculation, more detailed comparative studies were undertaken, particularly with Prices' tobacco ring-spot No. 2 (3), Imle and Samson's tomato ring-spot (1), and Zaumeyer's alfalfa-mosaic virus (6). These viruses were secured either directly or indirectly from the above-named investigators.

SYMPTOMS

The symptoms of the broad ring-spot disease are not sufficiently distinctive to permit satisfactory or reliable identification of the causative virus on this basis alone. The relatively wide band of chlorotic or necrotic tissue comprising the circumference of the ring is, compared to other ring spots, often distinct, but the variability is too great to allow one to attribute much diagnostic significance to this character.

On young tobacco plants (variety Havana No. 38), the first symptoms on inoculated leaves appear as indistinct yellowish spots in 4 to 6 days.

[1] Supported in part by allotments from the University of Wisconsin Research Fund.

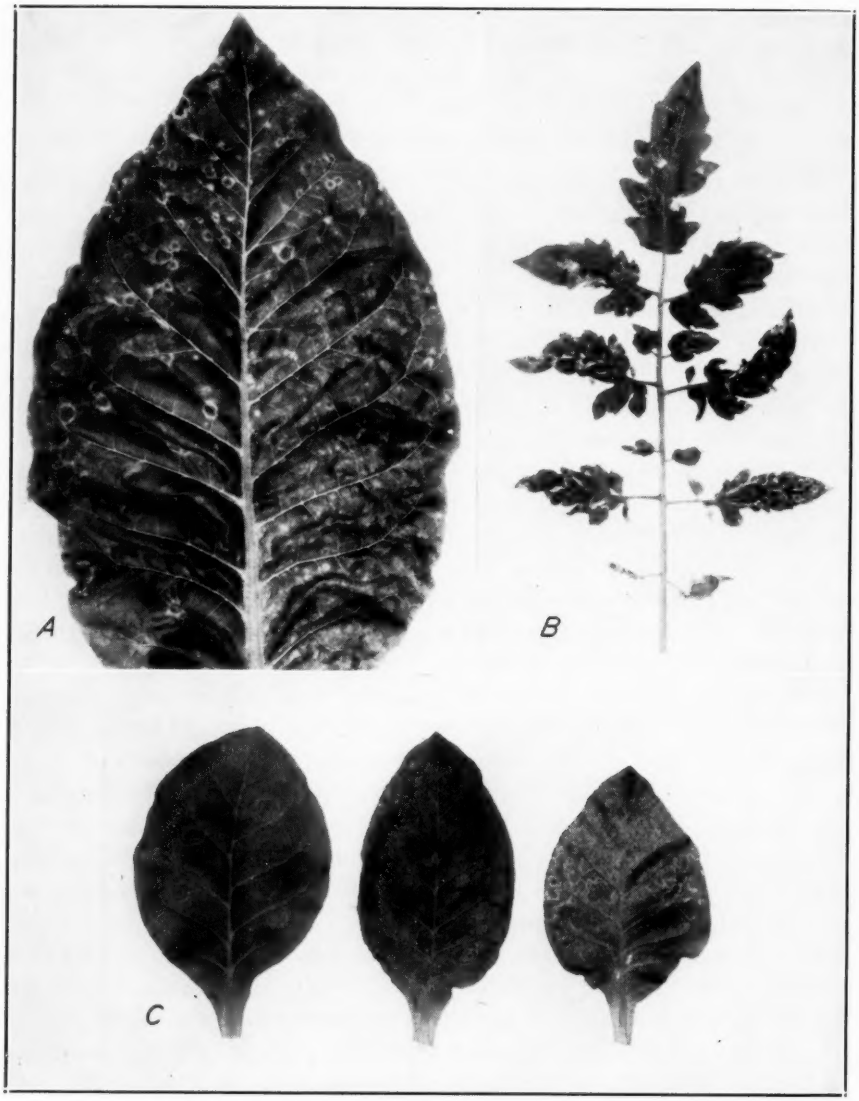

FIG. 1. *A*. A tobacco leaf from the original collection of broad ring spot from the field. These symptoms were characteristic on about 30 per cent of the plants in a 6-acre field. *B*. Artificial inoculations of broad ring spot to tomato under greenhouse conditions yield characteristic but less distinct ring-spot symptoms and considerable distortion. *C*. Artificial inoculations with broad ring-spot virus by the wiping method produces few but good inoculative symptoms on tobacco (leaf at left) but more abundant and distinct systemic symptoms (leaves at center and right).

These local areas may show a few fine, concentric, necrotic lines (Fig. 1, *C*), but more often the lesions become chlorotic rings. Systemic symptoms develop in about 7 days. The young, systemically infected leaves may at first become slightly puckered along the veins, resulting in some malformation (Fig. 2, *A*). Small chlorotic rings then appear, often 2 or more being ar-

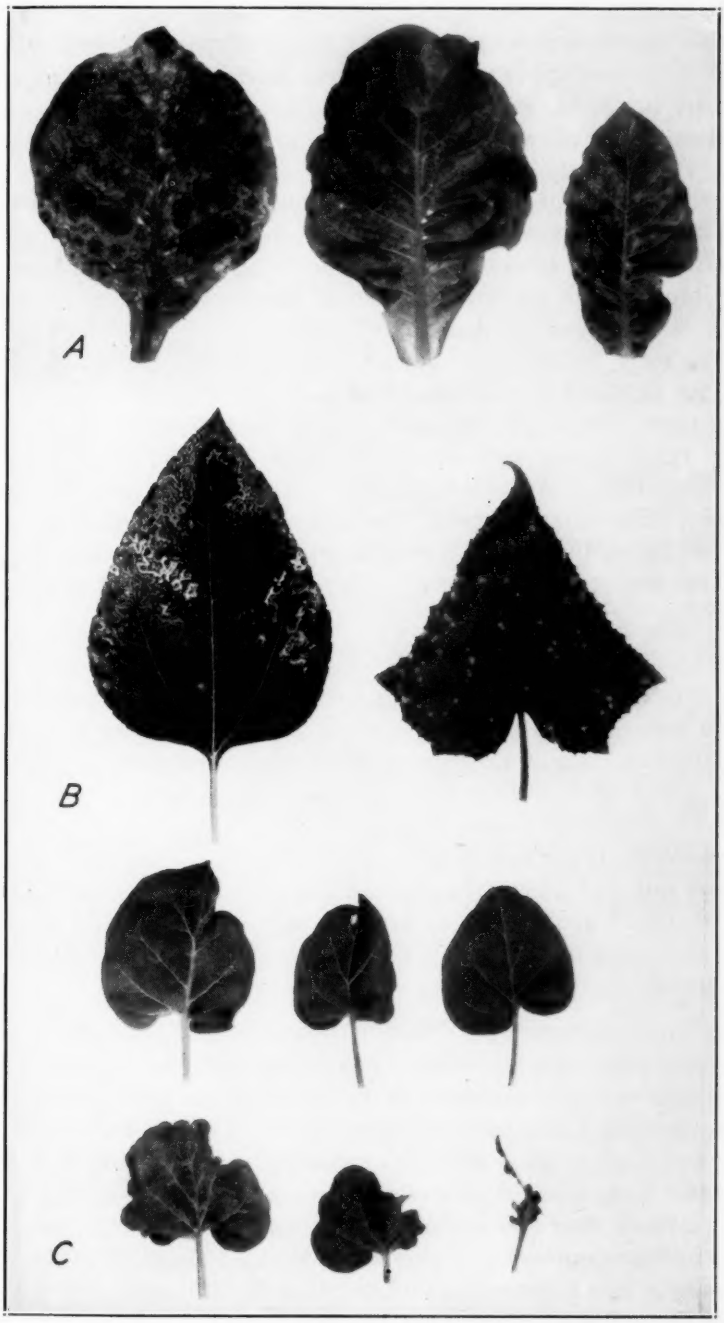

FIG. 2. *A*. Broad ring-spot virus often produces some puckering and malformation on systemically infected tobacco leaves. *B*. Systemic symptoms of broad ring spot on artificially inoculated sunflower (left) and cucumber (right) leaves. *C*. The symptoms of broad ring spot on *Nicotiana glutinosa* are not characteristic of those on other hosts, but the leaves are often variously deformed as shown here.

ranged concentrically (Fig. 1, *A*). Their location seems to bear no definite relation to the veins, as contrasted to ordinary tobacco ring-spot symptoms. Systemic symptoms appear on 2 or 3 young leaves. Succeeding new leaves, as they are produced, are apparently healthy. This recovery, however, is not permanent. Following the development of 3 or 4 apparently healthy leaves, it is not unusual for a young leaf to again develop symptoms especially on the tip half of the leaf. It has been shown also that the virus is present in the leaves showing no symptoms. Such leaves do not yield symptoms at the point of inoculation with broad ring-spot virus, although they may be infected with the viruses of potato ring spot, ordinary tobacco ring spot, tobacco ring spot No. 2, and alfalfa mosaic.

In the early fall and late spring, when greenhouse temperatures are higher, the chlorotic type of ring spot is partly replaced by fine, brown, necrotic rings. This type of symptom was characteristic also of the appearance of the disease in the field (Fig. 1, *A*). On tomato (variety John Baer), the virus also induces definite chlorotic and necrotic ring-spot symptoms. The rings, however, are somewhat broader than on tobacco leaves, and follow the veins to a greater extent (Fig. 1, *B*). Tomato leaflets usually are distorted by the virus to a much greater extent than are tobacco leaves.

TRANSMISSION OF THE VIRUS

The broad ring-spot virus is easily transmitted by mechanical inoculation. In some early trials, higher percentages of infection were obtained with diluted extracts of the virus if the leaves to be inoculated were first lightly dusted with carborundum powder. Consequently, carborundum powder was used in all property studies.

No extensive trials have been made in an effort to find insect vectors of the broad ring-spot virus. In 7 trials, however, involving the transfer of several thousand aphids (*Myzus persicae* Sulz.) from diseased plants to a total of 35 healthy tobacco plants no transmission of the virus by this insect has occurred.

PROPERTIES OF THE VIRUS

Property trials were made on the fresh extract of diseased tobacco plants. In table 1 is shown a summary of the data on the thermal death-point, longevity *in vitro*, and tolerance to dilution.

Thermal death-point determinations were made by heating 1 cc. of fresh leaf extract in a thin-walled test tube immersed in a water bath for 10 minutes. The virus never survived 54° C. for 10 minutes, but occasionally remained infective after being held at 52° C. for 10 minutes. The thermal death point is therefore about 54° rather than 52° C. as stated in the earlier abstract (2). The virus survived for 42 hours, but not for 48 hours, *in vitro* at room temperature. In other trials, extracts held at 4° C. or frozen at about −2° C. retained their infectivity for 16 days. Extracts diluted to 1:1000 with tap water occasionally caused infection, while greater dilutions

TABLE 1

Summarized results from tests on certain physical properties of the tobacco broad ring-spot virus on tobacco

Thermal death-point

Temp. °C. of exposure of virus for 10 min.	None	48°	50°	52°	54°
No. plants inoculated	55	45	55	55	45
No. plants infected	55	22	12	6	0

Longevity *in vitro*

No. of hours of aging of virus at room temperature	None	30	36	42	48
No. plants inoculated	70	35	70	55	30
No. plants infected	70	20	15	10	0

Tolerance to dilution

Amount of dilution of virus extract with water	None	1:250	1:500	1:750	1:1000	1:2500
No. plants inoculated	50	20	40	40	45	25
No. plants infected	50	15	16	14	6	0

did not. The dilution end point is therefore beyond 1:1000 instead of about 1:750, as stated in the preliminary abstract (2).

Filtration trials were made on extracts partly clarified by low-speed centrifugation, followed by dilution with an equal volume of distilled water or buffer solution. In 4 trials, with the extract at the normal pH of 6.3, adjusted to pH 4.8 and again to pH 8.0, the virus failed to pass a Berkefeld V filter. The use of Berkefeld N, Chamberland F filters, and Seitz E. K. filter pads in other tests also gave negative results. The unfiltered control extracts gave 100 per cent infection in all instances. The broad ring-spot virus, therefore, is evidently not readily, if at all, filterable.

HOST RANGE OF THE VIRUS

Actively growing young plants of many plant species have been inoculated with the broad ring-spot virus. Seven to 14 days after inoculation, whether or not symptoms were secured, subinoculations were made back to healthy tobacco plants. The virus was reisolated from the following species distributed through the families shown:

AMARANTHACEAE: *Amaranthus retroflexus* L.; BORAGINACEAE: *Cynoglossum amabile* Stapf. and Drum.; CHENOPODIACEAE: *Chenopodium album* L., *Spinacia oleracea* L.; COMPOSITAE: *Calendula officinalis* L., *Callistephus chinensis* Nees., *Helianthus annuus* L., *Zinnia elegans* Jacq.; CRUCIFERAE: *Brassica arvensis* Kuntze, *B. nigra* Koch, *B. oleracea* L., *Bursa bursa-pastoris* (L.) Britton; CUCURBITACEAE: *Cucumis sativus* L., *Cucurbita maxima* Duchesne; EUPHORBIACEAE: *Acalypha virginica* L.; LEGUMINOSAE: *Dolichos lablab* L., *Phaseolus vulgaris* L., *Pisum sativum* L., *Trifolium incarnatum* L.,

Vigna sinensis Endl.; MALVACEAE: *Hibiscus esculentus* L.; POLYGONACEAE: *Fagopyrum esculentum* Gaertn., *Rumex crispus* L.; PORTULACACEAE: *Portulaca oleracea* L.; RANUNCULACEAE: *Delphinium* sp.; SCROPHULARIACEAE: *Antirrhinum majus* L.; SOLANACEAE: *Datura stramonium* L., *Lycopersicum esculentum* Mill., *Nicandra physalodes* (L.) Pers., *Nicotiana angustifolia* Ruiz. and Pav., *N. glutinosa* L., *N. rustica* L., *N. sylvestris* Speg. and Comes., *N. tabacum* L., *Petunia violacea* Lindl., *Physalis* sp., *Solanum melongena* L., *S. nigrum* L., *S. tuberosum* L.; TROPAEOLACEAE: *Tropaeolum majus* L.; UMBELLIFERAE: *Apium graveolens* L.

Symptoms of broad ring spot varied from pronounced chlorotic rings, as on sunflower (Fig. 2, *B*, left), to scattered yellow spots, as on cucumber (Fig. 2, *B*, right). The more usual type of symptom on the majority of species was, however, a mild vermiculate pattern of chlorosis. *Nicotiana glutinosa* (Fig. 2, *C*) and *N. sylvestris* leaves were much distorted. Infections on squash and potato remained localized, but the virus became systemic on all other hosts tested under greenhouse conditions. Some of the hosts, although yielding definite symptoms of broad ring spot, permitted only with much difficulty transfer of the virus back to tobacco. Such sources of poor inoculum are represented by *Cucumus sativus, Datura stramonium, Solanum melongena, Chenopodium album,* and *Spinacia oleracea.*

No symptoms developed and no virus could be recovered from inoculated plants of *Beta vulgaris* L., *Citrullus vulgaris* Schrad., *Medicago sativa* L., and *Melilotus alba* Desr.

DISCUSSION

On the basis of symptoms only, broad ring spot of tobacco might easily be confused with other virus diseases of the ring-spot type. The wide host range of this virus also bears some resemblance to those of ordinary tobacco ring-spot (5), tobacco ring-spot No. 2 (4), and the alfalfa-mosaic (6) viruses. There are however some significant differences, as, for example, on tomato and spinach, which are both susceptible to the broad ring-spot virus by mechanical inoculation, but the former host is not susceptible to either ordinary tobacco ring-spot or alfalfa-mosaic virus, and the latter host is not susceptible to tobacco ring-spot No. 2 virus by the same method of inoculation. Significant differences also exist in the physical properties of the above-named viruses, particularly in the thermal death point, which is somewhat lower for the broad ring-spot virus than for either of the tobacco ring-spots or the alfalfa-mosaic viruses. These factors, together with the proven failure of these viruses to yield any cross immunity in the plants, may be regarded as sufficient evidence that broad ring-spot virus is a new one rather than a virus strain, although it is perhaps much more closely related to the viruses with which it has been compared in this investigation than to any other known viruses.

The rather unusual circumstance of the virus being found only once under natural conditions and then in comparative abundance, as described

in the introduction of this paper, suggests many possible interesting interpretations.

There is little probability in our opinion that the virus arose in Wisconsin by variation from ordinary tobacco ring-spot or other viruses of tobacco. The most logical explanation is that the virus existed previously in some unknown host plant in the vicinity of the seed beds or the field and was transmitted to the tobacco plants either mechanically or by an unknown and perhaps rare insect vector. It also may be assumed that the combination of circumstances that brought about the 1938 epidemic was due to a rare proximity of the hosts involved, or that the new virus cannot or has not yet become established on tobacco as a new host because of still other environmental conditions.

The discovery of new or undescribed viruses in nature, in field crops, or under greenhouse experimental conditions is now somewhat frequent, largely because more attention is given to the subject. It is quite likely that a serious attempt to locate and determine new viruses in nature, disregarding their frequency or economic importance, would yield a considerable number of new and distinct forms. The finding of new strains of known viruses is less significant because the origin of such variants is well established.

The problem of the origin of viruses may be perhaps more advantageously investigated with such rare viruses as broad ring-spot than with such a commonly prevalent form as the ordinary tobacco-mosaic virus. Either the numerous viruses already known (and perhaps a greater number yet to be determined) have come down through the ages, or are being developed more currently, descent and evolution being independent of any genetic mechanism yet defined.

SUMMARY

A description is presented of a new virus found on tobacco in the field. It has been given the common name "broad ring-spot" to distinguish it from other viruses producing similar types of symptoms.

The virus is transmissible to several other plant families, its thermal death-point is 54° C., maximum longevity *in vitro* about 42 hours, and the dilution end point slightly above 1 in 1000. It is apparently not filterable by ordinary methods, and is not transmitted by the common peach aphid. The properties and other characteristics are sufficient to distinguish the broad ring-spot virus from certain other viruses with which it has been compared.

The broad ring-spot virus, although too rare to be of any practical significance, is of some special interest to those working on problems relating to the origin and to the distribution of viruses in nature.

WISCONSIN AGRICULTURAL EXPERIMENT STATION
AND DIVISION OF TOBACCO INVESTIGATIONS,
UNITED STATES DEPARTMENT OF AGRICULTURE.

LITERATURE CITED

1. IMLE, E. P., and R. W. SAMSON. Studies on a ring-spot type of virus on tomato. (Abstract) Phytopath. **27**: 132. 1937.
2. JOHNSON, JAMES and R. W. FULTON. A rare virus disease on tobacco. (Abstract) Phytopath. **30**: 13. 1940.
3. PRICE, W. C. Specificity of acquired immunity from tobacco ring-spot diseases. Phytopath. **26**: 665–675. 1936.
4. ————. Comparative host ranges of six plant viruses. Amer. Jour. Bot. **27**: 530–541. 1940.
5. WINGARD, S. A. Hosts and symptoms of ring-spot, a virus disease of plants. Jour. Agr. Res. [U. S.] **37**: 127–153. 1928.
6. ZAUMEYER, W. J. A streak disease of peas and its relation to several strains of alfalfa mosaic virus. Jour. Agr. Res. [U. S.] **56**: 747–772. 1938.

THE GENUS CEREBELLA CESATI—ITS BIOLOGIC STATUS AND USE

R. F. Langdon

(Accepted for publication August 20, 1941)

The genus *Cerebella* was described by Cesati in 1851 from material collected on *Bothriochloa ischaemum* in Northern Italy, and was for many years regarded as a true smut, closely allied to *Thecaphora* or *Urocystis*. In 1902 Soccardo transferred the genus to the Deuteromycetes, but the true relationship between *Cerebella* and the host plant has not been recognized by a number of workers in various countries, who have regarded *Cerebella* as pathogenic and in itself responsible for damage to the spikelets of grasses. Others have recognized the association of *Cerebella* with the honey-dew (sphacelia) stage of ergot, on which it grows saprophytically, forming a dark-brown or black convoluted stroma between the glumes of the grass spikelets. Cesati apparently realized the association of *Cerebella* with the ergot of *Bothriochloa* (due to *Claviceps pusilla*, described by Cesati in 1861 after Tulasne's elucidation of the life history of *Claviceps* in 1853), for, in his description of the genus *Cerebella*, is the phrase ". . . *verisimiliter ex sclerotio sphaceligeno oritur.*" Subrmaniam (21), in describing a number of new species of *Cerebella* from India, mentioned that the genus was commonly found associated with species of *Sphacelia* on Gramineae. But, from his remark that one species of *Cerebella* produced black swellings on the grain of *Sorghum vulgare*, and the fact that he described a separate species for each host plant, it seems that he had no clear idea of the true relationship between *Cerebella* and the plants on which it is found. Recently Subramaniam has reported that all *Cerebella*-infected plants examined in 1921 showed also the conidia of ergot.[1]

McDonald (14) reported that an experimental plot of *Sorghum* in Kenya in 1923 was attacked by *Cerebella sorghi-vulgaris*, which had not previously been reported from Africa. Later, he announced that inoculation experiments with *Cerebella* on native sorghum had given positive results, but only when the heads were exuding a sugary secretion. This had been observed only in cold weather, and McDonald was uncertain whether or not it was due entirely to climate (15). There is but little doubt that the "sugary secretion" observed by McDonald was the sphacelia stage of a species of *Claviceps*, a disease known on *Sorghum* in India (18) and Africa (23). Wilkinson (24) regarded *Cerebella* as "a potential disease of cultivated graminaceous crops" in Kenya in 1926. Ajrekar (1) concluded that "*Cerebella* grows as an epiphyte or parasite on the sphacelia stromata or sclerotia of *Claviceps*"; but Rhind (19) recognized *Cerebella* as a saprophyte of ergot honeydew. Gonçalvez (10) has recently reported *Cerebella* associated with the sphacelia of *Claviceps* on several hosts in Brazil, and he considered that *Cerebella* was parasitizing the ergot and not the higher host plant.

[1] Privately communicated.

From Kenya, Gold Coast, and Southern Rhodesia there are several records of diseases of grasses, such as false smut and ear mould, said to be due to *Cerebella*, with no suggestion of any association with ergot (16, 6, 12, 13). Recently, Hopkins[2] has stated that, although *Cerebella* on various grasses is listed among the diseases of Southern Rhodesia, it has always been recognized there as a saprophyte. But yeast, *Fusarium*, and *Cladosporium* are found as saprophytes of ergot honey-dew too, and there is little justification for listing *Cerebella* instead of *Sphacelia* sp. or *Claviceps* sp. in host indexes. Hansford (11), in a list of diseases in Uganda, records *Cerebella* as a disease of several species of grasses, and for some other grasses mentions an association of *Cerebella* with *Sphacelia* sp. In practically every case the host given for *Cerebella* or some closely related host species has been recorded as ergotized elsewhere.

In Queensland, *Cerebella* was first recorded in 1887 when *Cerebella paspali* Cke. and Mass. (*Cerebella inquinans* (Berk. and Br.) Sacc.) was described from *Paspalum orbiculare* Forst. (*P. scrobiculatum* L., of early Australian authors), the fungus then being regarded as a smut allied to *Urocystis* or *Thecaphora* (8). Later, *Cerebella* was recorded from *Themeda australis* and *Heteropogon contortus* (4). The only other Queensland records of *Cerebella* are to be found in the Herbarium of the Plant Pathology Section of the Queensland Department of Agriculture, where several specimens having an encrustation of *Cerebella* on the glumes have been labelled "Smut," and in one case, *Thecaphora* sp. All of these specimens have shown ergot conidia beneath the superficial *Cerebella* when teased up in water on a slide.

Since May, 1940, collections of *Cerebella* on 13 species of grass have been made in southeastern Queensland, and an examination of these has shown that the conidia of *Claviceps* are always present in abundance in the same spikelet as the *Cerebella*. The same host species also have been found ergotized and quite uncontaminated with *Cerebella*. *Cerebella* from each host has been isolated and grown in pure culture on potato-dextrose agar where the characteristic cerebriform stroma of the genus is produced within a few days. No morphological differences between the isolations from 13 different hosts have been detected, the species being determined as *Cerebella inquinans* (Berk. and Br.) Sacc. This is in marked contrast to the 8 species of *Cerebella* recorded from 15 host species by Subramaniam. In culture *Cerebella* grows well on potato slices and on potato agar containing sucrose, glucose, or honey, the fungus producing spores readily on each of these. Inoculation of *Paspalum dilatatum* and *P. orbiculare* with a suspension of spores of *Cerebella* taken from a pure culture gave negative results; but inoculation of *P. dilatatum* with a mixture of conidia of *Claviceps paspali* and *Cerebella inquinans* produced an exudation of honey-dew after seven days, and the typical stromata of *Cerebella* appeared on the ergotized spikelets 2–3 days later. When a suspension of *Cerebella* spores in diluted honey was used for inoculation, a very slight superficial growth of that fungus

[2] Privately communicated.

occurred on the glumes, but there was no sign of parasitism by *Cerebella* and no interference with seed production.

Taking the evidence from the literature in conjunction with the results quoted above, it can be said that *Cerebella* is no more than a saprophyte that finds any carbohydrate-rich substratum suitable for growth. In this connection it is interesting to note that, in Brazil, *Cerebella* sp. has been found accompanying the attacks of the coffee-berry borer (3). In Europe, *Cerebella italica* is known to occur on the seeds of the chestnut and the oak, and *C. negeri* on the seeds of *Abies* (20).

No evidence has been found of any parasitism of *Claviceps* spp. by *Cerebella*, as suggested by Ajrekar (1) and Gonçalvez (10). In the very few cases seen where *Cerebella* was present after sclerotium development began, the *Cerebella* was found to be quite independent of the sclerotium and flourished only round the edges of the glumes, where a certain amount of the sugary secretion remained. In the majority of *Cerebella*-infected plants, development of sclerotia had not taken place before the spikelets were overgrown by the saprophyte.

The need exists for a revision of the species of *Cerebella*, of which, very probably, too many have been described. Ellis and Everhart (9), in describing *Cerebella spartinae* (regarded by them as a smut) from the United States (U.S.A.), refer to the stromata on *Spartina* as "subconfluent, extending along one side of the spike," and they remark that *C. spartinae* differs from *C. andropogonis* in habit. Differences in shape and position of stromata must be expected in a fungus that grows on ergot honey-dew that may be smeared about on the inflorescence. Other workers have followed Subramaniam, and, where possible, have determined their species of *Cerebella* according to the host plant on which it is found, *e.g.*, *C. panici* on *Panicum*, *C. cynodontis* on *Cynodon*. The arbitrary nature of this practice usually is recognized, but until the genus is monographed and good specific characters established, precise specific names cannot be applied in every case. Probably a number of the 18 species of *Cerebella* that have been described will be found to be synonymous with the early species, such as *C. andropogonis* and *C. inquinans*.

DISCUSSION

Several important points arise from an exact knowledge of the relation existing between the higher host plant and *Cerebella*.

Firstly, *Cerebella* should be removed from the host indexes, *e.g.*, the host indexes of India (7), of Queensland (5), of Southern Rhodesia[3] (12), and of Uganda (11), and *Sphacelia* sp. substituted, followed by a serious attempt to discover which species of *Claviceps* is responsible. (This is now being done in Southern Rhodesia and Queensland.) It is evident that there is a wide host range of ergot in Africa, India, and southeastern Asia; and the possibility of introducing new species of ergot to Australia or other countries in imported seed must not be overlooked. The record of *Cerebella andropogonis* on *Brachiaria* sp. by the U. S. Department of Agriculture Plant

[3] See footnote 2.

Quarantine and Control Administration (2) serves to emphasize the necessity for carefully examining imported seeds to prevent the introduction of ergot. Where *Cerebella* is seen, it is a warning against ergot, which may not be detected if the sclerotia are small and similar in shape to the seeds.

Secondly, *Cerebella* provides a natural control of ergot by inhibiting sclerotia formation, a fact already noted by Rhind (19) and Ajrekar (1). Early in the season *Cerebella* is not much in evidence, but, from January onwards, it has been observed in ever-increasing abundance on *Paspalum dilatatum;* it is also very common on *Bothriochloa* spp. and *Dichanthium* spp., which are attacked by *Claviceps pusilla* Ces. in Queensland. On an area of about half an acre of *P. dilatatum* at Kangaroo Point, Brisbane, almost every ergotized spikelet is now (April, 1941) infected by *Cerebella,* and it is practically impossible to find sclerotia on the area. A restriction of sclerotia formation to early summer, at least in some seasons, must be an important means of reducing the amount of inoculum available in the next spring, and may, to some extent, determine whether or not an epiphytotic of ergot will occur in the following season. Cessation of development of *Claviceps* following *Cerebella* infection is probably due to the effect of "staling" products produced during the growth of the *Cerebella* in the honey-dew.

Thirdly, *Cerebella* is a good field indicator of the presence of ergot. Recently a number of new ergot hosts have been found in Queensland, several of which might have been overlooked were it not for the conspicuous growth of *Cerebella* on the inflorescences. Bulk collection of inflorescences in the neighborhood of *Cerebella*-infected plants usually provides plenty of sclerotia and honey-dew of *Claviceps* for further study.

Fourthly, the records of *Cerebella* provide valuable evidence concerning the history of ergot in Queensland and other countries. *C. anthaenantiae, C. panici* and *C. sorghi* were described as smuts by Tracy and Earle in America in 1899 (22), and in view of the known occurrence of ergot on cultivated and native species of *Sorghum* and on native grasses in Africa, India, and Australia, a knowledge of the ergot of *Anthaenantia, Panicum* and *Sorghum* in America would be of considerable interest. Of the ergotized native grasses recently found in Queensland, there are records of *Cerebella* on *Themeda australis,* and *Heteropogon contortus* in 1890 (4), and on *Paspalum orbiculare* in 1887 (8). Specimens of *Cymbopogon refractus* and *Bothriochloa decipiens,* collected in 1911 and 1912, respectively, are in the Herbarium of the Plant Pathologist, Department of Agriculture, Queensland. Both of these are infected with *Cerebella,* but show also conidia of *Claviceps,* though the former was previously identified as *Thecaphora* sp. These records show that the ergot of these grasses is of long standing in Queensland.

SUMMARY

The literature on the genus *Cerebella* has been reviewed, and further evidence is presented to show that *Cerebella* is a saprophyte of carbohydrate-

rich materials, the commonest substratum for the fungus being the sugary honey-dew secretion associated with the sphacelia stage of *Claviceps* spp. The need for a revision of the species of *Cerebella* is pointed out.

Four points arise from a knowledge of the relation between *Cerebella* and the plants on which it is found:

1. *Cerebella* should be removed from the host indexes, and *Sphacelia* sp. substituted.

2. *Cerebella* provides a natural control of ergot by inhibiting the development of sclerotia.

3. *Cerebella* is a good field indicator of the presence of ergot.

4. The history of ergot in a country may be traced through old records of *Cerebella* on grasses.

DEPARTMENT OF BIOLOGY,
UNIVERSITY OF QUEENSLAND, BRISBANE, AUSTRALIA.

LITERATURE CITED

1. AJREKAR, S. L. Observations on a disease of Jowar (*Sorghum vulgare*) caused by *Sphacelia* (conidial stage of *Claviceps*). Jour. Indian Bot. Soc. **5**: 55–61. 1926.
2. ANONYMOUS. List of intercepted plants. U.S.D.A. Plant Quar. and Control Admin. Rev. App. Mycol. **11**: 544. 1932.
3. AVERNA-SACCA, R. The pathological manifestations which accompany the development of the borer *Stephanoderes hampei* on the berries or seeds of coffee. Rev. App. Mycol. **5**: 422. 1926.
4. BAILEY, F. M. Synopsis Qld. Flora, 3rd Supp., p. 123. 1890.
5. ———. Comp. Cat. Qld. Plants, p. 771. 1909.
6. BUNTING, R. H. Fungi affecting graminaceous plants of the Gold Coast. Gold Coast Dept. Agr. Bull. **10**: 51 pp. 1928.
7. BUTLER, E. J, and G. R. BISBY. The Fungi of India. Imp. Counc. Agr. Res. Sci. Monogr. **1**: 237. 1931.
8. COOKE, M. C., and G. MASSEE. Two remarkable fungi. Grevillea **16**: 20. 1887.
9. ELLIS, J. B., and B. M. EVERHART. New species of N. American fungi from various localities. Proc. Acad. Nat. Sci. (Phila.). 1893.
10. GONÇALVEZ, R. D. *Cerebella andropogonis* Cesati. parasitando *Claviceps* sp. sobre varias gramineas. Biologico São Paulo **3**: 2, 74–75. 1937.
11. Hansford, C. G. Annotated host list of Uganda parasitic fungi and plant diseases. E. Afr. Jour. Agr. **3**: 319–324. 1938.
12. HOPKINS, J. C. F. A list of plant diseases occurring in southern Rhodesia. Rhod. Agr. Jour. **29**: 462–467. 1932.
13. ———. A descriptive list of plant diseases of southern Rhodesia, and their control. Mem. Dept. Agr. S. Rhod. **2**: 1–51. 1939.
14. MCDONALD, J. Report of the Mycologist. Ann. Report Dept. Agr. Kenya, p. 83. 1923.
15. ———. Report of the Mycologist. Ann. Report Dept. Agr. Kenya, p. 145. 1925.
16. ———. Report of the Mycologist. Ann. Report Dept. Agr. Kenya, p. 229. 1927.
17. ———. Report of the Mycologist. Ann. Report Dept. Agr. Kenya, p. 134. 1932.
18. MCRAE, W. Madras Agr. Dept. Yearbook (**1917**): 109–110. 1917.
19. RHIND, D. Mycological notes on Burma. Int. Rev. Agr. (n.s.) **29**: 8, 744. 1928.
20. SACCARDO, P. A. Sylloge Fungorum, **25**: 583. 1931.
21. SUBRAMANIAM, L. S. The genus *Cerebella* in India. Jour. Proc. Asiatic Soc. Bengal, (n.s.) **17**: 205–208. 1921.
22. TRACY, S. M., and F. S. EARLE. New fungi from Mississippi. Bull. Torr. Bot. Club, **24**: 494. 1899.
23. WALLACE, G. B. A revised list of plant diseases in Tanganyika Territory. E. Afr. Jour. Agr. **2**: 305. 1937.
24. WILKINSON, H. Report of the Mycologist. Ann. Report Dept. Agr. Kenya, p. 155. 1926.

A QUANTITATIVE METHOD FOR ASSAY OF TOBACCO-MOSAIC VIRUS PROTEIN

VERNON L. FRAMPTON

(Accepted for publication September 19, 1941)

For the most part the various workers interested in the serological aspects of the virus problem have been content with qualitative observations, or at best quasi-quantitative observations, even though it has been shown by Heidelberger and his coworkers, in a long series of papers published in the *Journal of Experimental Medicine*, that the reaction between an antibody and its antigen satisfies the criteria for quantitative chemical analysis. Kleczkowski[1] has recently shown that, in a general way, the serological behavior of tobacco-mosaic virus, of aucuba-mosaic virus, and of bushy-stunt virus is in each case typical of the behavior Heidelberger has observed in his work with various other proteins. The data presented herewith were obtained in another connection where the quantitative aspects of serology were not the prime consideration, but they serve to show that the reaction between tobacco-mosaic virus protein and its antibody may be subject to quantitative treatment, and in that respect serve as additional evidence in support of some of the views that Heidelberger holds, and they confirm some of the observations reported by Kleczkowski. The main consideration, however, is that they give us a convenient, rapid and reliable method of quantitative assay in studies involving small quantities of this particular virus protein. The method unquestionably may be extended to several other plant viruses.

The serum used in these studies was obtained in the usual manner by the intervenous injection of small quantities of the isolated virus protein, suspended in 0.87 per cent sodium chloride solution, into a rabbit at intervals of 3 days for several weeks. The serum, obtained by bleeding the anesthetized animal, was stored in a frozen condition after the red cells had been centrifuged out.

The virus protein used in the precipitin reaction was an electrodialyzed preparation of the isolated protein which was suspended in water to which enough sodium chloride had been added to give an 0.87 per cent solution. The dissolution of the antigen in 0.87 per cent NaCl solution is essential in quantitative work involving the precipitin reaction. The reason for this precaution is that the antibody is in the globulin fraction of the serum, and this particular serum fraction is precipitated unless the salt concentration is maintained in the various reactions in which it may be involved.

In the precipitin reaction carried out in these studies, 1 cc. of serum was added to a 25-cc. volumetric flask, and then a definite volume of the virus suspension was added, and finally enough 0.87 per cent NaCl solution was added to bring the volume up to the mark. The flask contents were

[1] Kleczkowski, A. Quantitative studies on the serological reactions of some plant viruses and of a pea nodule bacterium (*Rhizobium leguminosarum*). Br. Jour. Exp. Path. 22: 44–58. 1941.

thoroughly mixed and the flask was placed in an incubator at 37° C. for 24 hours. The antibody content of the reaction mixture was thus held constant and in no case was the virus protein added in sufficient quantity to completely deplete the antibody supply. That is, the antibody supply was always in excess. Thymol was used as a preservative.

The first determinations were nephelometric. In nephelometry and in colorimetry the transmission of light by a solution or suspension depends on the thickness, d, of the layer traversed, and on the concentration, c, of the colored component of the solution, or of the suspension. The quantitative expression relating these factors is known as Beer's law, and is written

$$I_d = I\, e^{-kdc}$$

where I is the intensity of the incident light and I_d is the intensity of the transmitted light.

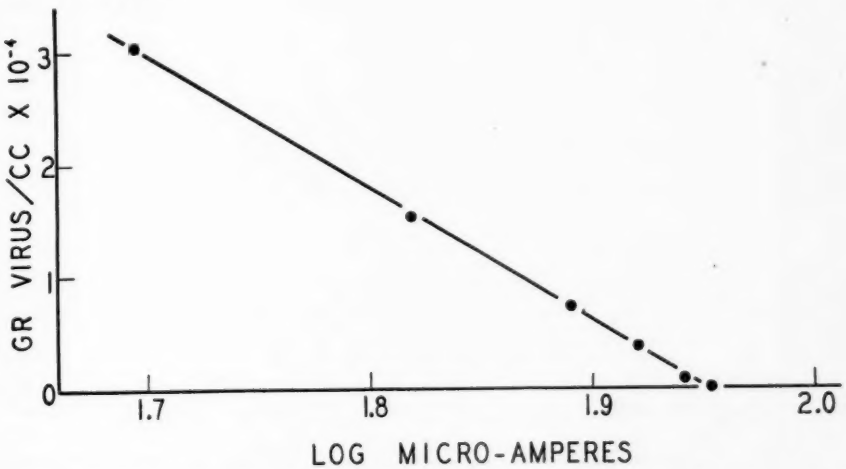

FIG. 1. The relationship between the amount of virus protein added to the serum in the reaction vessel and the nephelometric reading. Ordinate is the quantity of virus added to the serum; the abscissa is the current density observed in the nephelometer.

In nephelometry and colorimetry, as practiced, using the new photoelectric colorimeters, one varies c, keeping d constant, and observes the effect of that variation on I_d. If log I_d varies directly with c, where c is built up in the absorption cell of the colorimeter or nephelometer, one may conclude that Beer's law is followed. Beer's law has been found to be valid for many cases, but exceptions are known. Invariably, where exceptions to Beer's law are encountered in colorimetry, it has been found that the systems giving rise to the exceptions are systems where association or disassociation occurs, and where the degree of association or disassociation depends on the concentration. Difficulties in nephelometry are encountered when the precipitate formed in the absorption cell settles out too rapidly, or where the rate of growth of crystals or aggregates, once the nuclei have been formed, is great. Given the situation, however, where Beer's law is followed, one may conclude without much hesitancy that the reaction involved in the absorption

chamber, giving rise to the component that is being measured, is stoichiometric. One may state in this connection that, although the virus protein particles do disassociate in dilute solution, the degree of dissociation in the dilutions studied does not seem to be great.

In the work reported in this paper, the conditions were such that the current output of the photronic cell in our colorimeter was proportional to the light intensity. In figure 1, the logarithm of the current density observed in the nephelometric determination is plotted against the quantity of virus protein added to the serum in the reaction vessel. The conclusion is obvious, and the nephelometric technique as outlined may serve as an accurate and rapid means of assay.

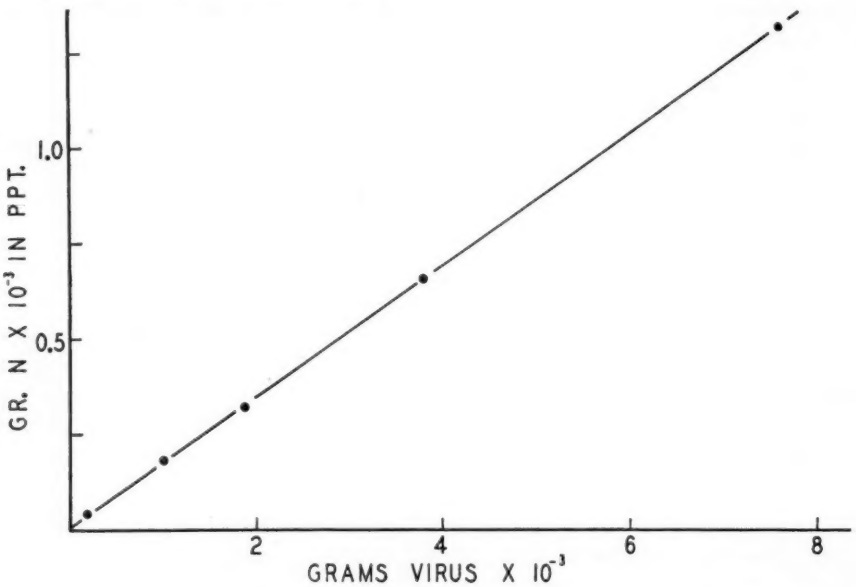

FIG. 2. The weight of nitrogen found in the precipitate formed in the precipitin reaction is plotted against the quantity of virus added to the serum in the reaction vessel.

Nitrogen and phosphorus determinations on the precipitate obtained from the precipitan reaction substantiate the view that, in the region of antibody excess, the reaction between the antigen and antibody is stoichiometric. The determinations were made in each case on the precipitate that had been packed in the bottom of a centrifuge tube and had been washed 3 times by suspension in 0.87 per cent NaCl solution, with subsequent sedimentation in the centrifuge. Nitrogen was determined by means of the Kjeldahl procedure. Data are presented in figure 2. The amount of nitrogen found in the precipitate is proportional to the amount of virus protein added to the reaction vessel.

Phosphorus determinations were made according to the Eddy and Deeds[2] method. Digestion was effected by means of a small quantity of H_2SO_4,

[2] Eddy, C. W., and Floyd Deeds. A photoelectric method for the determination of phosphorus. Ind. and Eng. Chem. 9: 12–14. 1937.

together with a few drops of H_2O_2 (table 1). The quantities of phosphorus in these samples was very small, and consequently the error in phosphorus determinations was large, but the data indicate that the precipitation of the virus is substantially complete, and that there is no P in the antibody. One would conclude from these data that, in the region of antibody: antigen ratios studied, for each part of virus protein nitrogen in the precipitate there are 0.21 parts of antibody nitrogen. The ratio of 0.21 was obtained from the consideration that the ratio of phosphorus to nitrogen in the virus protein is 1:40, together with the analytical data for nitrogen and phosphorus in the precipitate. Kleczkowski reported a ratio of 0.25.

TABLE 1.—*Phosphorus determinations from samples of virus precipitate as per the Eddy-Deeds method. Digestion was effected by adding H_2SO_4 and a few drops of H_2O_2*

G. virus protein used	G. P. found	G. P. expected[a]	Per cent recovery
7.6×10^{-3}	27×10^{-6}	30×10^{-6}	90
3.8	13	15	87
1.9	7.1	7.6	93

[a] G. of phosphorus expected on the basis of a determination of 0.4 per cent as the phosphorus content of the virus protein and on the assumption that precipitation of virus protein by the antibody is complete, and on the assumption that the antibody contained no phosphorus.

The following procedure is recommended in using serology in virus assay in the case of tobacco mosaic virus protein, where one is dealing with plant sap: Adjust pH of 50 ml. of virus-containing sap to some value between 4.5 and 3.5, and filter through a few grams diatomaceous earth (it has been observed that the adsorption on diatomaceous earth is complete at pH 4.5 or less). The filtrate is discarded. Suspend the filter cake in 25 ml. of water, cautiously adjust pH to 8.5, using NaOH, mix thoroughly and filter. Wash the filter cake at least 3 times with a few cc. of water. Combine the filtrate and washings in a 100-ml. volumetric flask, add enough NaCl to give 0.87 per cent solution, and finally add enough 0.87 per cent NaCl solution to bring the volume up to the mark, then mix. Add 1 ml. of serum containing the antivirus to 24 ml. of the virus extract, and after thoroughly mixing, store at 37° C. for 24 hours. In the event that the color originally present in the plant sap has not been entirely eliminated, centrifuge the virus-antivirus mixture strongly ($3{,}000 \times G$) for about 30 minutes, decant, and suspend the precipitate in a known volume of 0.87 per cent NaCl solution. The last traces of color may be eliminated by a repetition of the sedimentation. The suspension is then placed in the nephelometer, and the reading obtained is referred to the calibration curve, where one may read off directly the amount of virus protein in the cell of the nephelometer.

The calibration curve is obtained by adding progressive amounts of the virus protein suspended in 0.87 per cent NaCl to successive volumetric flasks, each of which contains the same amount of serum, say 1 ml., and then bringing the volume up to the mark by means of 0.87 per cent NaCl solution. Mix the flask contents and store at 37° C. for 24 hours. Stir up the sedi-

ment before pouring the suspension into the nephelometer cell. The log of nephelometer reading is plotted against the amount of virus added to the volumetric flasks.

In the event that a suitable nephelometer is not available, or in the event color cannot be removed, virus protein assay that is more satisfactory than the local lesions method may be obtained from phosphorus determinations made on the washed precipitates, following the method of Eddy and Deeds and using an ordinary colorimeter. This suggestion comes from the consideration that the infectivity of the isolated virus preparations, as determined by the local lesions method, may vary from preparation to preparation by as much as 8000 per cent.

Several suggestions may be in order concerning quantitative serological work with the viruses in conjunction with the nephelometric technique. Where it is possible, the isolated virus protein should be used as the antigen. One reason for this suggestion is that complications may arise as a consequence of injecting unknown material into the experimental animal. By the way of an example, there is frequently enough solanin present in the sap of potato foliage to cause a fatal hemolysis if the whole sap is used for injection, and, at best, a non-fatal hemolysis results in a discolored and unsatisfactory product. A second reason for the suggestion that the isolated virus protein be used as the antigen is that during the manipulation of the protein involving the use of concentrated salt solutions and filtrations, the microorganisms originally present are largely eliminated, and the danger of septicemia is thereby reduced. A third reason for the suggestion is that one may then be in a position to take advantage of the specificity of the precipitin reaction and determine the virus protein content of a sample irrespective of its contamination with other proteins (neglecting cross reactions). In addition, the picture will not be complicated by the presence of a mixture of unknown antibodies.

It is recommended, finally, that a separate calibration curve be obtained for each serum sample.

SUMMARY

The precipitin reaction may be used in a rapid and accurate determination of tobacco-mosaic virus protein.

The amount of nitrogen and phosphorus found in the precipitate formed in the precipitin reaction is proportional to the amount of virus protein added.

The ratio of virus protein nitrogen to antibody nitrogen in the precipitate is 4.8.

CORNELL UNIVERSITY,
ITHACA, NEW YORK.

A METHOD OF GROWING PLANTS IN WATER VAPOR TO FACILITATE EXAMINATION OF ROOTS[1]

WALTER CARTER

(Accepted for publication October 11, 1941)

INTRODUCTION

In studies requiring the frequent and critical examination of roots, considerable mechanical injury and interference with growth result when the plants are grown in soil or even in sand and aerated water culture. The method of growing pineapple plants described herein was devised in order to facilitate the examination of the roots following infestation of the plants by mealy bugs.

THE WATER VAPOR METHOD

This consists of setting the plants in the perforated top of a simply constructed wooden box, in which is fixed an atomizing device similar to

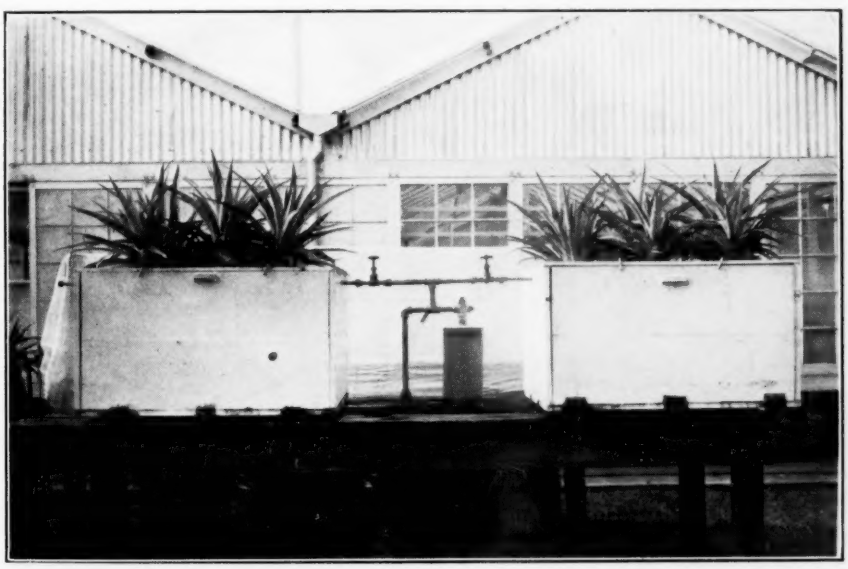

FIG. 1. Water-vapor culture box planted May 15, 1941, and photographed August 18, 1941.

those devices used to keep vegetables fresh when they are displayed for sale, or in air-conditioning apparatus. These devices permit a very fine jet of water to impinge on a metal plate, which results in a steady drift of finely divided vapor. Domestic water pressure is adequate.

To improve rooting and growth, a cylinder to contain concentrated nutrient solution is included in the system. For the 12 plants shown in figure 1,

[1] Published with the approval of the Acting Director as Miscellaneous Paper No. 37 of the Pineapple Research Institute of Hawaii, University of Hawaii.

this cylinder, which contains 5 liters of the nutrient solution, is refilled every other day. The nutrient solution used is made up in the following proportions with the addition of an accessory solution of minor elements:

	Cc. of ½ molar stock solution
KH_2PO_4	400
$Ca(NO_3)_2$	900
$MgSO_4$	450
$(NH_4)_2SO_4$	50
K_2SO_4	400

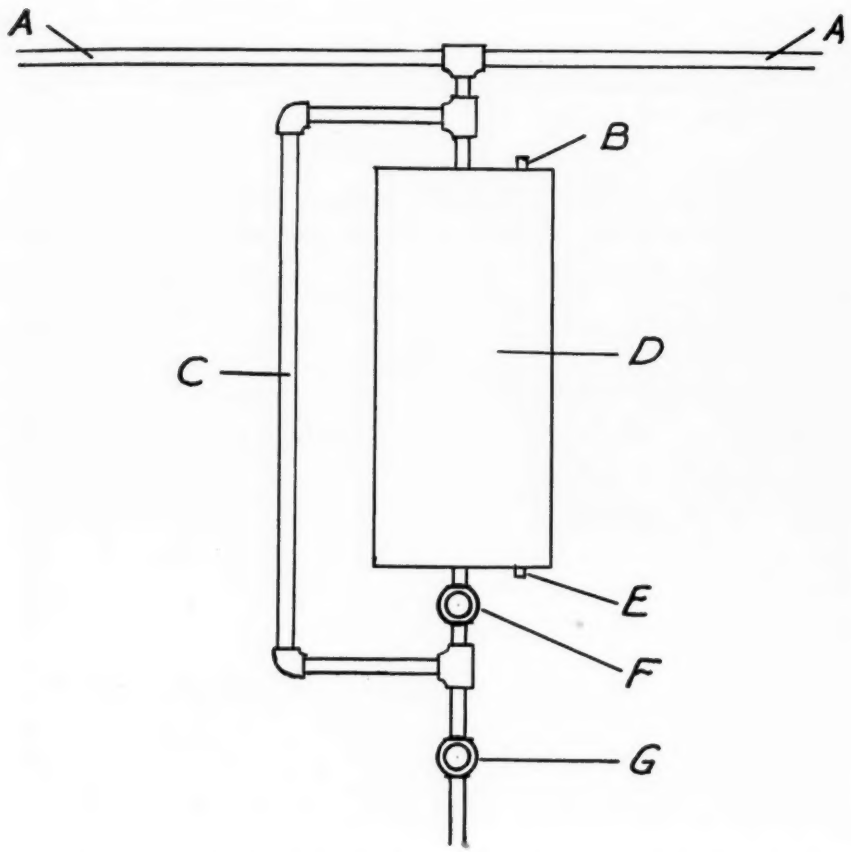

FIG. 2. Diagram showing device for introduction of nutrient solution into the water stream. A, to vapor boxes; B, filling plug; C, by-pass at full pressure; D, nutrient solution tank; E, drain plug; F, needle valve; and G, water supply valve.

This system does not provide a nutrient of constant concentration, but rather one that gradually dilutes. The discharge of nutrient from the cylinder was improved, following a suggestion by C. T. Schmidt,[2] when the water supply, instead of being led directly through the cylinder, is arranged as shown in figure 2. One arm of the T connection in the water line by-

[2] Associate Entomologist, P.R.I.

passes the water at full pressure around the cylinder; the other arm of the T connection leads to the cylinder, and the volume of water passing through the cylinder is reduced by means of a needle-point valve. In this way the exhaustion of the nutrient solution is usefully delayed.

Pineapple plants grown from vegetative slips by this method have grown at least as vigorously and as rapidly as in sand and water cultures in which the same nutrient solution was used. Root development is extensive (Fig. 3). The roots can be readily inspected and samples taken for study without damaging the balance of the root system.

FIG. 3. Root development on 3-month-old plants in the water vapor culture box, August, 1941.

The vigor of growth obtained suggests the possibility of extending the use of this method, not only to the study of roots but also as a modification of the numerous methods employed in the water culture of commercial plants.

PINEAPPLE RESEARCH INSTITUTE OF HAWAII,
HONOLULU, HAWAII.

CHLOROPICRIN AND ETHYLENE DICHLORIDE FOR ROOT-KNOT NEMATODE CONTROL

A. G. Newhall and F. L. Stark, Jr.

(Accepted for publication November 22, 1941)

In the short space of 10 years, chloropicrin has become the top-ranking chemical for treatment of soils infested with nematodes. We are indebted to a score or more of investigators for proving its efficacy and developing techniques for employing it. Particular attention might be called to the work of Johnson and Godfrey (5), Johnson (4), Neller and Allison (6), Godfrey (2), Newton, Bosher and Hastings (8), Howard, Stark and Smith (3), Young (10), Chitwood (1), and Taylor and McBeth (9), who have all tested it for root-knot control (*Heterodera marioni*).

Chitwood (1) recently pointed out that ethylene dichloride alone and in suitable mixtures with chloropicrin could be used at less expense and inconvenience to the operator than undiluted chloropicrin. Therefore, the authors compared chloropicrin alone at 2 cc. per injection with ethylene dichloride at 15 cc. and with a 1–9 mixture of the two at 10 cc. The work was done in a ground bed of a greenhouse built over fine sandy loam near Rochester, New York. The root knot had been progressively increasing in severity for several years, so that in 1940 only 2.75 lb. of tomatoes per plant were obtained from the spring crop, which is considered less than half of normal. The growers were about to try chloropicrin before installing steaming equipment, and readily agreed to turn over for experimentation an area of 1500 sq. ft. in one end of a 200-foot house, 30 ft. wide.

On September 26, 1940, 4 weeks after removal of the previous crop of tomato vines, 4 replicate plots of each of the 3 treatments plus 4 checks each $7\frac{3}{4} \times 12$ ft. in extent were laid out in a 4×4 Latin square (Table 1). The

TABLE 1.—*Plot arrangement and root scores indicating degree of nematode injury 11 months after treatment of soil with chloropicrin (Cp), ethylene dichloride (Ed), and a 1–9 mixture of these (Mix). N = no treatment*

N—59	Cp— 8	Mix— 2	Ed—33
Mix— 4	Ed—30	N—42	Cp—11
Cp— 9	Mix— 0	Ed—19	N—56
Ed—33	N—42	Cp— 5	Mix— 7

loose sandy soil was slightly moist and contained many partly decayed root galls of variable size up to $\frac{1}{2}$ in. in diameter. Soil temperature at a 3-in. depth was 67° F. Injections were made 4 to 5 in. deep with a "Larvajector"[1] at 10-in. intervals in rows 10 in. apart. After the injections were made and the holes tamped by stepping on them, water was liberally

[1] Hand-operated injector, holding approximately 2 quarts, made by Innis, Speiden & Co., Niagara Falls, N. Y.

sprinkled on all plots to seal the gas beneath the surface. In a similar manner the grower employed chloropicrin to soil in the remainder of the house and in another larger one.

Radishes were sown on the experimental plots November 23. These were harvested in February and were followed later by tomatoes. Yield records on the radish crop were kindly furnished the writers by the grower.[2] But, as might be expected from the fact that this is a low-temperature crop, no evidence of nematode activity was observed nor any significant differences found in total yields, due to treatments, as measured by the number of bunches harvested from each plot. The growers, however, noted that the radishes reached a stage large enough to harvest a few days sooner on the plots treated with the 1–9 mixture of chloropicrin and ethylene dichloride than on the others.

FIG. 1. Roots selected as standards by which to score those from the experimental plots. They represent (left to right) classes 4, 3, 2, 1, and 0.

On April 20, 1941, young tomato plants were set throughout the house 12 or 15 in. apart in rows approximately 2 ft. apart, so that 6 rows of 6 or 7 plants occupied each plot. Unfortunately, it was impracticable to obtain yield records of the tomatoes on the individual plots, but, by mid-summer, the check plots could easily be denoted by the stunted growth and yellowish color of these plants and conspicuous nematode galls on many of their surface roots.

All vines were loosened with a fork, pulled up, and carefully examined for nematode injury on August 23. The following scheme was employed to evaluate the different treatments for root-knot control. Individual plants, showing various degrees of nematode injury, were first selected for standards and placed in 5 classes ranging from no infection, which was assigned a zero, to very severe infection, which was assigned a value of 4 (Fig. 1). Fifteen roots were then taken from the center of each plot. Each was scored according to these standards; for example, if a root had been injured to approximately the same extent as standard number 2, it was scored as 2.

[2] Acknowledgment is made to County Agent R. G. Palmer, for courtesies and help in carrying out this experiment.

Little difficulty was experienced in placing all 15 roots in the correct classes. The 15 scores for each plot were then totaled and this total considered as an estimation of the extent of nematode damage. The greater the amount of injury the larger the number. Applying these totals, it was possible to compare the relative values of the treatments for nematode control by employing the analysis of variance method for a Latin square. The results obtained are shown in tables 1 and 2.

It is evident that all treatments resulted in a very significant control of root-knot nematode. Moreover, chloropicrin alone and the mixture of chloropicrin with ethylene dichloride were superior to the ethylene dichloride alone. These differences all proved to be statistically significant with odds greater than 99:1. The slight difference in control between chloropicrin alone and the mixture did not prove to be statistically significant. The same conclusions would have been drawn on the basis of mere observation. (See Table 2 and Fig. 2.)

TABLE 2.—*Summary of root scores by treatments*

	No treatment	Ethylene dichloride, 15 cc. per injection	Chloropicrin, 2 cc. per injection	1–9 mixture of Cp. and Ed., 10 cc. per injection
Totals	198.0	115.0	33.0	13.0
Means	49.7	28.75	8.25	3.25

Least mean difference for significance, odds { 99:1 = 20.045 / 19:1 = 12.070

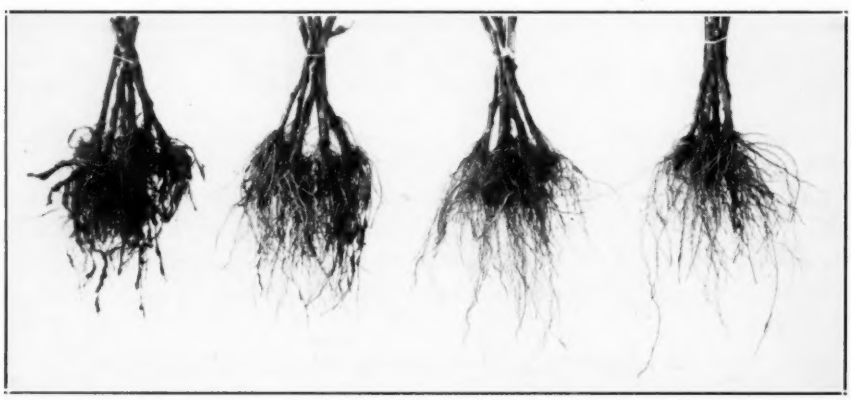

FIG. 2. Typical roots from plots receiving (left to right) no treatment, ethylene dichloride 15 cc., chloropicrin 2 cc., and the 1 to 9 mixture at 10 cc. per injection.

DISCUSSION

Some additional evidence of the value of the chloropicrin treatment was obtained from the growers' experience. As previously stated, they treated the remainder of the houses (14,000 sq. ft.) with chloropicrin in the same manner and at approximately the same rate as in the experiment. Before

treatment their yields had dropped to a low of 2¾ lb. per plant in 1940 (less than half of normal for a spring crop). After soil treatment the 1941 crop yielded 7 lb. per plant. Although admittedly it is unscientific to compare the yield of one year with that of another, in this case there was no other factor that could presumably account for more than a small proportion of this 170 per cent increase in yield. Examination of the roots of over 1,000 plants revealed but very few nematode galls.

As judged by the amount of gall development on the experimental plots at the end of the season and by appearance of the plants during the last month, ethylene dichloride gave inferior control of nematodes to that obtained by either chloropicrin or the 1–9 mixture. However, without yield data we lack proof that ethylene dichloride is not economical, for Howard *et al.* (3) found that complete eradication of nematodes was not essential for normal yields of tomatoes. All that can be said now is that, until the present advance in price has receded, ethylene dichloride is definitely not recommendable. Final judgment should perhaps await further work on its fungicidal efficiency, which the writers believe is less than that of chloropicrin.

The cost of treatments may be summarized as follows: chloropicrin with 2-cc. injections 10″ × 10″ apart requires a calculated 10.5 lb. per 1000 sq. ft. At 80 cents a pound[3] the cost of material for 1000 sq. ft. would be $8.40 or .84 ct. per sq. ft. ($366 an acre). Ethylene dichloride, using 15-cc. injections, requires a calculated 61 lb. per 1000 sq. ft., which, at the 1939 price of 6.5 ct. per lb.[4] would be only $3.97, or .38 ct. per sq. ft. ($166 an acre). However, at 1941 prices these figures for ethylene dichloride would have to be doubled. Similarly, by calculation, the 1–9 mixture (volume basis) of chloropicrin and ethylene dichloride used at 10 cc. for each injection calls for 5¼ lb. of chloropicrin and 36.6 lb. of ethylene dichloride per 1000 sq. ft. The cost amounted to $6.57 two years ago, but would now be closer to $8.94, or .89 ct. per sq. ft. ($388 an acre). In practice, 10 to 15 per cent should be added to all these figures to care for losses due to evaporation, to extra injections made around the margins, to inaccuracies in calibration of the injectors, to spilling during the many filling operations, and to frequent testing of the injectors.

For comparison, we may cite the costs of steaming, given by Newhall (7) as .65 to 2.0 ct. per sq. ft., depending on the method used and the depth of penetration. Much of the expense of steaming derives from cost of equipment and labor. In these two respects fumigation of soil by volatile liquids has distinct advantages.

It seemed noteworthy to the writers that plots treated in September, with no barriers between them, showed such definite margins the following August. No clearer proof is needed to demonstrate the slow rate of lateral movement of *Heterodera marioni* in sandy soil under greenhouse conditions.

[3] Price, in 180-pound cylinders, November, 1941.
[4] Price, in 55-gallon drum.

SUMMARY

A simple statistical method of evaluating volatile soil fumigants was used to compare certain nematocides against *Heterodera marioni*.

Excellent control of nematodes was obtained on a spring tomato crop by soil treatments the previous September with the following: (1) Chloropicrin at 10.5 lb. per 1000 sq. ft. (460 lb. per acre) applied at 10-inch intervals, at 2 cc. per injection, 4 to 5 in. deep; (2) A mixture of chloropicrin plus ethylene dichloride, 1–9 at 10 cc. per injection (230 lb. chloropicrin plus 1594 lb. ethylene dichloride per acre).

Less effective control was obtained by 15-cc. injections of straight ethylene dichloride, 2656 lb. per acre. It is concluded that at the recently advanced 1941 price of 12 ct. a pound ethylene dichloride is not recommendable either by itself or mixed with chloropicrin. At sufficiently lower prices its use as a substitute for half of the chloropicrin may be worth considering as an economy measure.

CORNELL UNIVERSITY, ITHACA, NEW YORK.

LITERATURE CITED

1. CHITWOOD, B. G. Soil treatments with volatile liquids for control of nematodes. Phytopath. **31**: 818–824. 1941.
2. GODFREY, G. H. Experiments on the control of root-knot nematode in the field with chloropicrin and other chemicals. Phytopath. **25**: 67–90. 1935.
3. HOWARD, F. L., F. L. STARK, and J. B. SMITH. Chemical control of nematodes in tomato greenhouses. (Abstr.) Phytopath. **29**: 11. 1939.
4. JOHNSON, M. O. The Pineapple. Paradise of the Pacific Press. (Honolulu.) 1935.
5. ———, and G. H. GODFREY. Chloropicrin for nematode control. Indus. & Engin. Chem. **24**: 311–313. 1932.
6. NELLER, J. R., and R. V. ALLISON. A machine for the subsurface treatment of soils with chloropicrin and with carbon bisulfide for nematode control under field conditions. Soil Sci. **40**: 173–179. 1935.
7. NEWHALL, A. G. Control of root-knot nematode in greenhouses. Ohio Agr. Exp. Stat. Bull. 451: 1–60. 1930.
8. NEWTON, WM., J. E. BOSHER, and R. J. HASTINGS. The treatment of glasshouse soils with chloropicrin for the control of *Heterodera marioni* (Cornu) Goodey, and other soil pathogens. Canad. Jour. Res. Sec. C, 15, **5**: 182–186. 1937.
9. TAYLOR, A. L., and C. W. MCBETH. Spot treatments with chloropicrin and ethylene dichloride for control of root knot. Proc. Helm. Soc. Wash. **8**: 53–55. 1941.
10. YOUNG, P. H. Soil fumigation with chloropicrin and carbon bisulfide to control tomato root knot and wilt. Phytopath. **30**: 860–865. 1940.

STRAWBERRY LEAF ROLL, A NEW DISEASE[1]

G. H. Berkeley[2] and A. G. Plakidas[3]

(Accepted for publication October 23, 1941)

In June, 1938, Premier strawberry plants with a rather "delicate" appearance and a pronounced rolling of leaves were observed in a plantation near St. Catharines, Ontario. As the symptoms in the field suggested none of the known diseases of strawberries, specimens were brought into the laboratory for further study. When these plants had been observed for some time under greenhouse conditions, it was recalled that in 1935 a Parson's Beauty with similar symptoms had been grafted to a healthy Royal Sovereign and that the disease had been transmitted to the Royal Sovereign and 3 of its runners, but that all the plants had died shortly thereafter. In September, 1940, the junior writer observed a similar disease at Geneva, New York, on seedlings U.S.D.A. No. 1631 and Geneva No. 9270, whilst examining the strawberry-breeding plots there, and notes were compared when the junior writer visited St. Catharines in October, 1940. Since the St. Catharines material had been lost in the meantime, and the junior writer was leaving New York for Louisiana, where the disease does not occur, it was considered advisable to publish this short note on strawberry leaf roll, giving a description of the disease and the limited experimental evidence concerning its nature.

SYMPTOMS

The plant as a whole has a "delicate" appearance, due to small leaves and spindly petioles. However, the most conspicuous symptom is the downward rolling of the leaflets, which is most pronounced in the basal portion (Fig. 1). In extreme cases the rolling is such that the opposite margins of the leaflet touch, or even overlap, thus forming a funnel-shape tube. The leaves on affected plants are pale green, smaller and narrower than normal leaves of the same variety, and the petioles are longer and spindlier. The surface of the leaves is ruffled and rugose and shows irregular chlorotic areas of various sizes. Affected plants are on the whole smaller than normal plants, but there is no pronounced stunting.

TRANSMISSION BY GRAFTING

At St. Catharines, Ont. Under greenhouse conditions 3 of the 4 Premier plants used for grafting made good growth and approached the appearance

[1] Cooperative project, Science Service, Department of Agriculture, Ottawa, and Louisiana Agricultural Experiment Station. Contribution No. 682 from the Division of Botany and Plant Pathology.

[2] Senior Pathologist-in-charge, Dominion Laboratory of Plant Pathology, St. Catharines, Ontario.

[3] Associate Plant Pathologist, Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana. The junior writer's part of the work was done while he was spending his sabbatical year at Cornell University.

of healthy plants, though they still maintained a "delicate" type of growth due to spindly petioles and smaller leaves. Runners from these 3 plants were grafted[4] to runners of 3 different clonal plants of *Fragaria virginiana*. In one case the union was not successful and, though it appeared to be successful in the other two cases, the abnormal condition was not transmitted by grafting. The fourth Premier plant made poor growth and produced spindly runners that were difficult to graft; in fact, some of the runners dried up shortly after grafting. Nevertheless, 4 runners from this Premier plant were grafted to runners from different clonal plants of *Fragaria virginiana*, with the result that one graft made on July 18, 1938, was successful in transmitting the diseased condition to one *F. virginiana* plant (Clone No. 21) and its two daughter plants, thus indicating that the disease was of virus type.

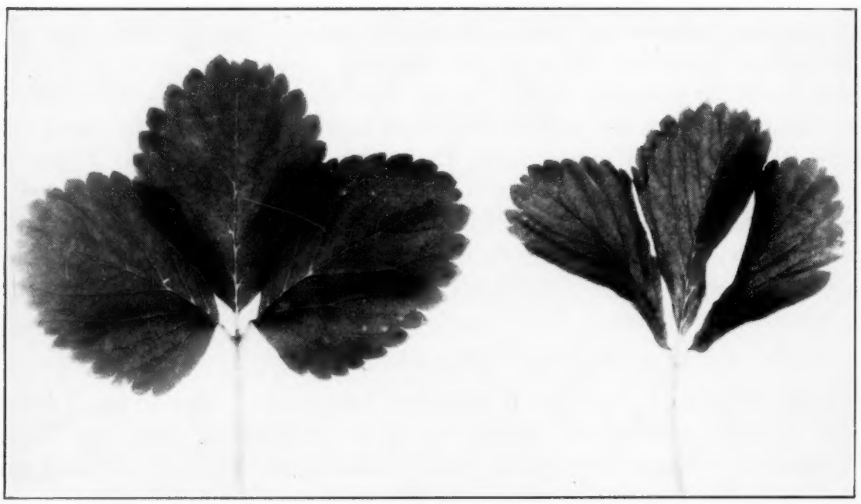

FIG. 1. Strawberry leaf roll. Comparison between healthy and diseased leaves of U.S.D.A. seedling No. 1631. × about ½ natural size.

At Cornell, N. Y. On October 4, 1940, 4 runner grafts were made, 3 between healthy and diseased plants of U.S.D.A. seedling No. 1631, and 1 between diseased and healthy plants of Geneva seedling No. 9270. The grafts were made at the terminal portion of the runners in such manner that the 2 runner-buds could be rooted in the same pot. At this time of the year runner growth was slow and it was doubtful whether the grafts would take. However, 2 of the 4 grafts made union, and in both these cases the disease was transmitted, thus corroborating the results obtained at St. Catharines. The first symptoms appeared 2½ months following grafting. In the other 2 grafts, in which union was not made, no transmission of the disease occurred, even though the healthy and diseased runners were rooted

[4] The grafting was done by A. A. Hildebrand, who was carrying on at the time a series of patch grafts in connection with studies on yellow-edge. For description of the grafting technique see "An Investigation on Strawberry Virus Disease in Ontario," by Harris, R. V., and A. A. Hildebrand. Can. Journ. Res., C, 15: 250–280. 1937.

in the same pot and the cut surfaces of their buds came into intimate contact.

CONCLUSION

Although the number of successful grafts was small, the evidence, nevertheless, indicates that strawberry leaf roll as described is a new, unrecorded virus disease of strawberries.

PHYTOPATHOLOGICAL NOTES

Banded Chlorosis, a Transmissible Disease of Cherry.[1]—In September, 1940, an interesting chlorotic pattern in the leaves of 2 varieties of Japanese flowering cherry was discovered. Further search for these symptoms indicates that they are commonly to be found in the Pacific Northwest on the 3 varieties of *Prunus serrulata* known as Amanogawa, Okochin, and Temari. Hundreds of cases of bud perpetuation were found in nurseries. This led to a further preliminary investigation of the disease, the leaf symptoms of which are rather variable.

There is one constant characteristic of the leaf symptoms. Whatever form the discolored area takes, it is bounded by a chlorotic band, usually about 1 to 2 mm. broad. The band may describe a circle, causing a ring spot or a chain of rings, usually between 2 lateral pinnate veins or along the leaf margin. In other cases more or less perfect "oak leaf" patterns are formed from the midvein and extending out to points on the lateral veins. In other cases a mere sector extending in from the leaf margin may be involved (Fig. 1). The chlorotic areas are whitish or yellowish, sometimes

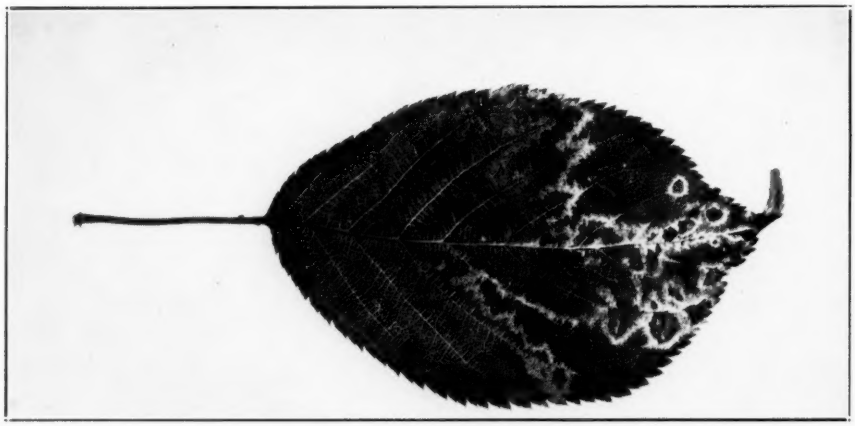

FIG. 1. A leaf of Amanogawa flowering cherry showing typical banded chlorosis.

becoming pinkish, in striking contrast to the green of the leaf. The only other apparent effect of the disease on flowering cherries was noted as a dieback of twigs of affected Amanogawa trees 16 or more years old. This dieback does not occur on young trees, and may not be a direct effect of the virus.

The first year's experiments with the disease involved the budding of 10 healthy mazzard seedling trees with diseased flowering-cherry buds of the Amanogawa variety. Ten seedlings were budded with healthy Amanogawa checks. The budding was done in September, 1940, and in the late spring of 1941; 9 of the budded trees (1 died) showed symptoms on the leaves of

[1] Published as Technical Paper No. 394, with the approval of the Director of the Oregon Experiment Station. Contribution from the Department of Botany.

the mazzard stocks. By summer the inoculated trees were dwarfed in comparison with the symptomless check trees. The disease, therefore, is not only transmissible to mazzard stock but also has a detrimental effect, especially on this stock. It was easily transmitted through budding, even though the buds themselves did not "take."

Many cases of transmission from flowering cherry to mazzard have been discovered this year in nurseries. Wherever there are mazzard sprouts from stocks supporting diseased Amanogawa tops, these sprouts usually show symptoms of banded chlorosis.

It is possible that dieback in older diseased trees of the Amanogawa variety may be due to the virus in the mazzard roots rather than the influence of the virus in the flowering-cherry cions.

Banded chlorosis, therefore, may be numbered among the transmissible viroses of *Prunus*. Rather elaborate studies of the disease are in progress to determine host range in the genus *Prunus*. As a binomial we suggest the name *Marmor pallidolimbatus* and "Prunus virus 10" in the numerical system.—S. M. ZELLER and J. A. MILBRATH, Oregon State College, Corvallis, Oregon.

Apothecia of Sclerotinia fructicola on Peach in Western Washington.—The occurrence of apothecia of *Sclerotinia fructicola* (Wint.) Rehm. has not been reported on peach in the State of Washington, according to the records in the plant-disease survey file in the Department of Plant Pathology at Pullman.

FIG. 1. Apothecia of *Sclerotinia fructicola* developing from a peach mummy.

In western Washington *Sclerotinia fructicola* causes considerable fruit decay of peach during the harvest period.[1] It may live over winter in mummies attached to trees and produce fresh moniliospores the following spring. Apothecia on mummies overwintering on the soil were not observed until the spring of 1941, although surveys in peach orchards had been made each spring for the four previous years. On March 26, 1941, apothecia were found developing from peach mummies beneath a tree in an orchard located in the Lake Shore district near Vancouver, Clark County, Washington (Fig. 1). A survey throughout the rest of that orchard and in other peach orchards of Clark County failed to reveal the presence of additional apothecia on peach mummies. Isolations were made from the apothecia. The cultural characters resembled those obtained from apothecia of *S. fructicola* isolated from Italian prune mummies. Inoculations with pure cultures of the organism caused fruit decay of both peach and Italian prune.—GLENN A. HUBER and KARL BAUR, Western Washington Experiment Station, Puyallup, Washington.

A Simple Technique for Aseptic Handling of Media.—The literature of phytopathology contains descriptions of techniques for measuring and dispensing sterile liquid culture media. Such methods usually call for a certain amount of apparatus of a sort not readily available in many laboratories.

Over a period of several years the writer has developed, and repeatedly used, a very simple method for dispensing sterile media to sterile flasks or adding sterile chemicals to previously sterilized flasks of media. The number of contaminations that have occurred in experiments in which this method has been employed is very small.

The technique is so simple that any person interested in employing it can readily make alterations of details to suit his own special needs. A brief outline follows:

1. Sterilize liquid culture media, vitamins, or other solutions, by filtration or other suitable methods.

2. Prepare empty culture flasks, or flasks of media to receive sterile chemicals, as follows: plug with cotton, make a cap of paper over the cotton (held on by a rubber band), and autoclave.

3. Plug with cotton, wrap in paper, and autoclave, a suitable number of graduate cylinders of suitable size. (50-cc. cylinders have been used extensively by the writer.) Each cylinder may be used safely for at least six to ten measurements.

4. Construct a transfer chamber. Arrange 4 or 6 ring stands, or two or three empty cartons, so as to form the outline of a chamber of the following dimensions: height, 20 inches; depth, 30 inches; and breadth, 30 inches. Cut pieces of cheese cloth, wet with 3 per cent lysol solution, and so hang them from the supports as to provide the chamber with walls and

[1] Huber, Glenn A., and Karl Baur. Brown rot on stone fruits in western Washington. Phytopath. 31: 718–731. 1941.

ceiling of wet cheese cloth. Sponge with Lysol the table and other exposed surfaces within the chamber. The front curtain must have sufficient slack to allow the worker's arms to perform under it.

5. Place all sterilized equipment, media, etc., in the chamber. Then, with an atomizer (drug store type), thoroughly spray the air of the chamber with a disinfectant solution. This removes any possible contaminating dust.

6. Just before entering the chamber wash the hands and arms with 70 per cent alcohol.

7. The removal of the paper caps from the flasks, or of the paper wrapping from the graduate cylinders, provides sterile, dust-free, cotton-plugged vessels. When the bacterial filter is removed from the filter flask of sterile medium, the lip is wiped with cotton soaked in 70 per cent alcohol. A sterile cotton plug is then transferred from an empty dummy flask to the filter flask, replacing the discarded filter apparatus.

8. Media may now be measured in a graduate cylinder and put into flasks without the use of a flame. Cotton plugs are handled in the usual manner. The plugs being sterile, and the air and the worker's hands comparatively germ-free, it is possible to make repeated measurements and transfers of media with no contamination. For adding 1-cc. portions of sterile solutions to flasks of sterile media, medicine droppers have been calibrated and sterilized in vials.

9. A desk lamp, inside the chamber, is a great aid to visibility through the cheese cloth. Turn out the ceiling lights in the room to reduce light reflection on the outside of the cloth.

The technique suggested is not considered superior to any others previously described in the literature, but may be useful in cases where equipment is very limited or where it is impractical to set up more elaborate apparatus.—J. ARTHUR HERRICK, Kent State University, Kent, Ohio.

A Nonpathogenic Buff-colored Barley Smut.[1]—Buffcolored chlamydospores of certain smut fungi have been found to cause infection on graminaceous hosts. To the writer's knowledge, however, the nonpathogenic type has not been described. Campagna[2] reported the occurrence in nature of a white loose smut on wheat. Holton,[3] in studies on hybridization between *Ustilago levis* (Kell. and Sw.) Magn. and *U. avenae* (Pers.) Jens., discovered a buff smut on oats, which originated by mutation in *U. levis*. The chlamydospores were smooth and colorless. Johnson, Rodenhiser, and Lefebvre,[4] in a study to determine the effect of incubation-period tempera-

[1] Cooperative investigation of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Idaho Agricultural Experiment Station. Published with approval of the Director of the Idaho Station as Research Paper No. 196.

[2] Campagna, E. New white smut collected at Sainte-Anne-de-la Pocatière. Ann. Rept. Que. Soc. Prot. Plants **18**: 71–72. 1926.

[3] Holton, C. S. Hybridization and segregation in the oat smuts. Phytopath. **21**: 835–842. 1931.

[4] Johnson, H. W., H. A. Rodenhiser, and C. L. Lefebvre. Two types of fall Panicum smut. Jour. Agr. Res. [U. S.] **61**: 865–875. 1941.

ture on the pathogenicity of *Sorosporium syntherismae* (Pk.) Farl., observed one smutted plant to have buff sori composed of hyaline, glabrous chlamydospores. Moore and Allison,[5] in 1934, found a single head of barley infected with what was described as an albino strain of *Ustilago hordei* (Pers.) Kell. and Sw. The head was almost white, and intermediate between the loose and covered smut type. The spores were colorless, glabrous, and smaller than those of *U. hordei*, and the germination was typical of the sporidium-forming smuts. Two sex groups were identified within the albino strain, monosporidial lines of which were compatible with those of the normal *U. hordei* and *U. medians*.

In September of 1936, the writer commenced a study of the genetics and

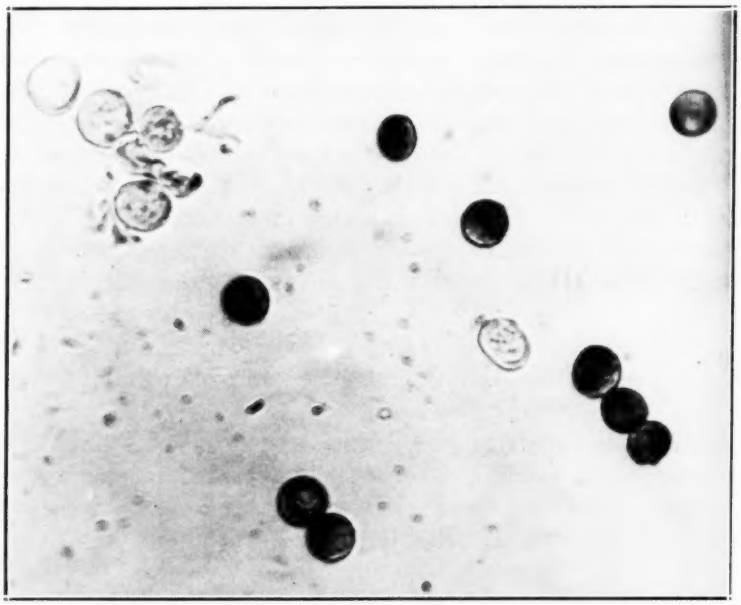

FIG. 1. Photomicrograph showing the contrast in color between chlamydospores of the buff smut of barley and those of the normal *Ustilago hordei*. ×900.

hybridization between a physiologic race of *Ustilago hordei* and two races of *U. nigra* Tapke. F_1 hybrid chlamydospores were obtained on Odessa barley (C.I. 934)[6] from infection with paired monosporidial lines of *U. hordei* and *U. nigra*. These chlamydospores and those of subsequent generations were collected from individual heads and used to reinoculate seed of the barley varieties, Nepal (C.I. 595), Lion (C.I. 923), and Himalaya (C.I. 1312). On one plant of Nepal there were 2 identical buff smutted heads that contained F_3 chlamydospores. Except for color, these buff smutted heads were identical with those containing the usual black chlamydospores of *U. hordei*.

[5] Moore, M. B., and C. C. Allison. An albino strain of barley smut. (Abstract) Phytopath. 25: 27–28. 1935.

[6] C.I. refers to accession number of the Division of Cereal Crops and Diseases, B.P.I., U. S. Department of Agriculture.

The spores were hyaline, glabrous, and apparently intermediate in size between *U. hordei* and *U. nigra* (Fig. 1). The sporidia were smaller than the sporidia of either *U. hordei* or *U. nigra,* but more nearly approached the *U. nigra* type; *i.e.,* they were long and narrow and somewhat pointed. Spore germination was irregular. Two or three sporidia instead of the expected 4 sporidia on a promycelium was rather common. The sporidia were difficult to detach from the promycelium. Fusion of sporidia in culture revealed 2 sex groups; however, pathogenicity tests using either chlamydospores or paired monosporidial lines failed to produce smut on Nepal or Odessa barley. This would indicate that the factors governing sex and pathogenicity are different.—WAYNE M. BEVER, College of Agriculture, University of Illinois, Urbana, Ill.

Heterocaryosis and Variability.—Some years ago, a paper[1] was published in which evidence was presented to show that monosporic cultures of *Botrytis cinerea* Pers. may contain genetically different nuclei. With the intent to clarify certain concepts of fungal variability, the writers pointed out that the term heterocaryosis precisely describes such a condition.

It has since become apparent that several writers have used this term in a restricted sense. For example, one author[2] states: ". . . each cell in both the mycelium and conidia of the Fusarium species contains one nucleus. . . . It is, therefore, concluded that heterocaryosis is not responsible for such saltations in these species." Another[3] writes: ". . . it seems very improbable that the induced sectoring in *H. sativum* race 1 was due to heterocaryosis." More recently, others state:[4] ". . . the fact that the vegetative cells are uninucleate seems to eliminate heterocaryosis as a cause of heritable variation in this organism."

From the above citations it appears that these writers either suspected heterocaryosis of being able to cause mutation or thought that such a view had been expressed in one of the earlier papers[1] on the subject. All agree in their conclusions that heterocaryosis is not the cause of mutation, two of them[2,4] appear to reach this conclusion on the basis that the cells of their organisms were uninucleate.

The writer agrees that heterocaryosis is not a cause of mutation. He also agrees that heterocaryosis cannot occur in uninucleate cells. In this connection, however, it is well to remember that at least two factors or mechanisms operate against the possibility of the uninucleate condition being continuous or permanent in any fungus. These two factors, mitosis and anastomosis, operating singly or together are able to produce binucleate,

[1] Hansen, H. N., and Ralph E. Smith. The mechanism of variation in imperfect fungi: *Botrytis cinerea.* Phytopath. 22: 953–964. 1932.

[2] Dickinson, Sydney. The nature of saltation in Fusarium and Helminthosporium. Minnesota Agr. Exp. Sta. Tech. Bull. 88. 42 pp. 1932.

[3] Christensen, J. J., and F. R. Davis. Variation in *Helminthosporium sativum* induced by a toxic substance produced by *Bacillus mesentaricus.* Phytopath. 30: 1017–1032. 1940.

[4] Keitt, G. W., and M. H. Langford. *Venturia inaequalis* (Cke.) Wint. I. A groundwork for genetic studies. Amer. Jour. Bot. 28: 805–820. 1941.

trinucleate and quadrinucleate cells in fungi generally considered to have uninucleate cells. The behavior of some such fungi when they are heterocaryotic has already been described.[5]

What is heterocaryosis? Hansen and Smith[1] state in a footnote that "The term 'heterocaryosis' precisely describes the condition of a cell containing two or more genetically different nuclei. . . ." It was not their intent that the term should or could be restricted as to where such a condition might occur. Heterocaryosis then is a term that exactly describes a *condition;* hence, the term may be used wherever such a condition exists not only in a single cell, but also when it exists in any or all cells of an individual thallus or organism. It may also be used in its adjective form to describe any structure in which the condition occurs. For example: the ascus of any heterothallic fungus is homocaryotic at one time and heterocaryotic at another. *Phoma terrestris* Han. has been isolated from nature only in the heterocaryotic condition.[5] A homocaryotic strain of a species of *Hypomyces* became heterocaryotic in culture by means of a mutation.[6]

How is heterocaryosis initiated? In two ways: (1) by mutation within a plurinucleate entity, and (2) by fusion or anastomoses between cells having genetically unlike nuclei.

How is heterocaryosis related to variability? It has been shown[5] that many fungi occur in nature in a heterocaryotic condition and when such fungi are isolated and worked with in the laboratory they may give the impression of great variability by producing tufts, patches, or sectors differing in appearance from the main part of the culture. In a fungus with multinucleate spores and two kinds of nuclei, variability appears to be even greater when many single spore cultures are made, for among these will be seen several distinct types, two of which are homocaryotic and the rest heterocaryotic. Among the latter may be present several culturally distinct types, because they contain different proportions of the two kinds of nuclei.[5] Heterocaryosis may, in this way, appear to be the cause of variability, whereas in reality, the basic cause of variability and the primary cause of heterocaryosis is mutation.—H. N. HANSEN, Division of Plant Pathology, University of California, Berkeley, California.

On the Cause of the Milo Disease.[1]—The cause of the root, crown, and shoot rot of milo, or Pythium root rot of milo, more commonly known as the "milo disease," according to investigations so far conducted, has been ascribed to *Pythium arrhenomanes* Drechs.[2,3] More recent studies begun by the writer in 1938 show that this sorghum disease is the result of a more

[5] Hansen, H. N. The dual phenomenon in imperfect fungi. Mycologia **30**: 442–455. 1938.

[6] Hansen, H. N., and W. C. Snyder. The origin and inheritance of *M* types in Hypomyces. Phytopath. **30**: 787. 1940.

[1] Contribution No. 430, Dept. of Botany, Kansas Agricultural Experiment Station.

[2] Elliott, Charlotte, L. E. Melchers, C. L. Lefebvre, and F. A. Wagner. Pythium root rot of milo. Jour. Agr. Res. [U. S.] **54**: 797–834. 1937.

[3] Kendrick, J. B., and F. N. Briggs. Pythium root rot of milo and the development of resistant varieties. Calif. Agr. Exp. Stat. Bull. 629. 1939.

complex set of factors than the literature states. The causal factors responsible for the typical symptoms shown by seedling and mature susceptible milo plants and milo hybrids in the greenhouse and field cannot be attributed to *P. arrhenomanes* alone.

When susceptible varieties and resistant selections of milos are grown in naturally infested soil in the greenhouse or field, susceptible varieties show very definite symptoms, followed by the death of the plants, while resistant selections and varieties remain alive and show marked resistance.[2] Recent investigations by the writer show that typical symptoms of the milo disease are not produced in the seedling stage in the greenhouse or in older plants in the field when grown in sterile or nonsterile soil containing pure cultures of *P. arrhenomanes*. Furthermore, it has been learned that this pathogen is equally pathogenic to susceptible milos and to the strains of milo and other common sorghums known to be resistant to the milo disease. Certain organisms, environmental conditions, or extenuating factors other than those studied in the investigations of this disease[2,3] are etiologically involved in the problem.

It is realized that the foregoing statements from an etiological point of view are largely negative, yet the facts represent information obtained in a study of the causal factors concerned in this disease. More detailed results of the present studies will appear in another paper. D. B. Creager and C. M. Slagg have assisted in these investigations.—L. E. MELCHERS, Dept. of Botany, Kansas Agricultural Experiment Station, Manhattan, Kansas.

Rhizoctonia Infection of Cotton and Symptoms Accompanying the Disease in Plants beyond the Seedling Stage.—Rhizoctonia (*Corticum vagum* Berk. and Curt.) is one of the causes of damping off of cotton seedlings. The attack usually occurs on seedlings in early planted cotton prior to the formation of true leaves. The pathogen invades the stem immediately below the soil line and causes the formation of dark to reddish-brown cankers. In severe attacks the cankers may encircle the stem and penetrate so deeply that the seedlings fall over and die. An unusual stage of the disease was observed in the Louisiana Delta in 1940 and 1941. The affected plants were in the early flowering stage, from 7 to 14 inches high. It was found that many of the plants were almost devoid of root systems especially lateral roots and were easily uprooted (Fig. 1, A). They were growing in a more or less prostrate condition and had few fruiting branches. Stems of affected plants had deep-seated cankers both above and below the soil line. Many showed typical "constrictions" that almost severed the stems a short distance below the surface of the soil (Fig. 1, B). Tissue cultures of infected plants yielded, in approximately 90 per cent of the platings, the Rhizoctonia fungus. It appears, therefore, that when cold, wet weather continues through late spring, the disease may persist and cause considerable injury to older cotton plants.—DAVID C. NEAL, Bureau of Plant Industry, U. S.

FIG. 1. Unusual symptoms of Rhizoctonia (*Corticium vagum*) infection of cotton. A. Delfos 425 plants infected with Rhizoctonia and approaching flowering stage. Note poorly developed root system, scarcity of lateral roots, and deep cankers on stems below soil line. B. Delfos 425 plants. Same age as those shown in A. Note cankers both above and below soil line and typical "constrictions" on stems. Plants collected and photographed on June 25, 1941.

Department of Agriculture and Louisiana Agricultural Experiment Station, Baton Rouge, Louisiana.

Olive Anthracnose in the United States.—A disease of the olive fruits was found on the Mission variety in December, 1941, on the campus of the University of California in Berkeley. The disease appeared to be quite similar to the olive anthracnose described in 1899 by d'Almeida[1] in Portugal. Initial symptoms of the disease consist of small brown spots on the ripe, as well as green, surfaces of unripe fruits. The lesions increase gradually in size, becoming irregular and depressed in shape. Later, the surface of the spots becomes brick-red in general appearance, owing to sporulation of the causal fungus, and in some cases turns black.

The olive anthracnose has been observed in France,[2] Spain,[3] Japan,[4]

[1] Almeida, J. V. d'. La "Gaffa" des olives en Portugal. Bull. Soc. Myc. France **15**: 90–94. 1899.

[2] Griffon, E., and A. Maublanc. Notes de pathologie végétale et animale. Bull. Soc. Myc. France **17**: 469. 1911.

Russia,[5] Greece,[6,7] and Uruguay.[8] Only in Portugal and in Greece has the disease caused important damage, reducing the quality of the fruits. In California also, it may become serious, particularly on olives used for oil production.

The fungus was readily isolated on potato-dextrose agar in Petri dishes by tissue plantings from the fruits and by direct transfer of the spores from

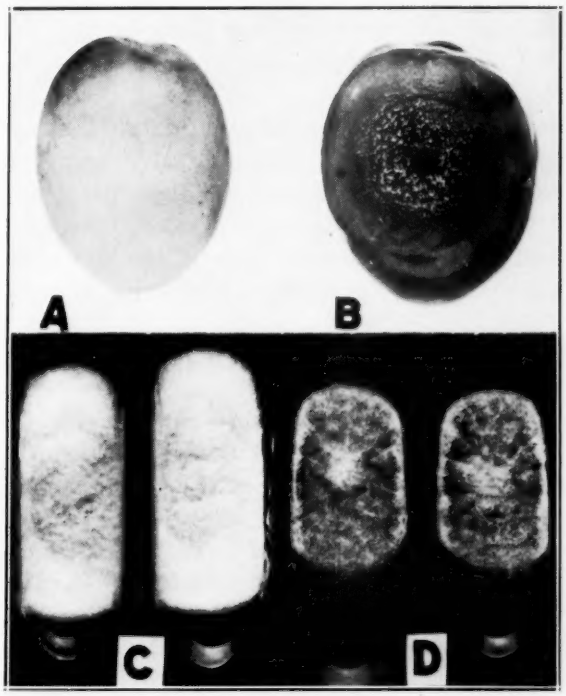

FIG. 1. A. Healthy fruit. B. Inoculated fruit. C. The mycelial type. D. The conidial type.

the lesions. The organism has been identified as *Gloesporium olivarum* Alm. Like many other imperfect fungi, this fungus exhibits the *dual phenomenon*,[9] that is, when single-spore cultures were made 2 types appeared: the mycelial

[3] Navarro and Nonell. Epifitias mas importantes del Olivo en España. VII Congres Int. Oleiculture. (Seville.) 311 pp. 1924.

[4] Hemmi, T., and S. Kurata. Contributions to the knowledge of anthracnose of plants. Jour. Soc. Trop. Agr. Taiwan. **6**: 573–583. 1935.

[5] Nagorny, P. I., and E. M. Eristavi. A brief survey of plant diseases in Abkhasia in 1928. Publications Agr. Exp. Sta. of Abkhasia 38. 28 pp. 1929. *In* The Rev. App. Myc. **9**: 326. 1930.

[6] Petri, L. Azione tossica dell' arsenito sodico sofra le spore del *Gloesporium olivarum* Alm. Boll. R. Staz. Pat. Veg. Ann. **10**: 359–361. 1930.

[7] Sarejanni, J. A. Catalogue commenté des champignons rencontrés sur les plantes cultivées en Grèce. Int. Phytopath. Benaki. (Athens) Ann. **3**: 60. 1939.

[8] Acosta, D. R. Investigaciones fitopatologicas. Min. Industrias. Direc. Agron. Publ. Mensual **4** (12): 1–18. 1932.

[9] Hansen, H. N. The dual phenomenon in imperfect fungi. Mycologia **30**: 442–455. 1938.

type producing abundant mycelium and few conidia, and the conidial type producing many conidia and less mycelium (Fig. 1, C, D).

Inoculations of wounded fruits of the Mission variety were made separately with spore suspensions of the conidial and of the mycelial types of the fungus. Symptoms were apparent after 36 hours, and 1 week later the acervuli appeared (Fig. 1, A, B). The 2 types that were reisolated proved to be identical with those obtained from the original cultures.

No great difference in virulence between the conidial and mycelial types could be detected as a result of these tests; both were pathogenic to the Mission variety.

A careful search of the literature indicates that this fungus has not previously been reported in the United States. Fruit specimens of the disease have been deposited in the herbarium of the Department of Botany, University of California, Berkeley.—RAFAEL E. PONTIS, Research Fellow of the Asociacion Argentina para el Progreso de las Ciencias, and H. N. HANSEN, Division of Plant Pathology, University of California, Berkeley.

Mycological Nomenclature.—The International Rules of Botanical Nomenclature are intended to give fixity to names, to avoid error, ambiguity, confusion, and the useless creation of names (Art. 4).

American Plant Pathologists have followed the spirit of these Rules. I hope they, and other mycologists, will give formal consideration to two changes I hereby propose, and that are being considered also in Britain:

1. That, *whenever necessary among Fungi, the conservation of specific names, firmly established in the literature by wide use over many years, be legalized.* For example, the specific epithets in the names *Tilletia tritici, T. laevis, Ustilago levis* and *Rhizopus nigricans* are illegitimate under the existing rules; their use will continue in any event, and should be legalized. Also, about a score of pre-Friesian names of Powdery Mildews used by Salmon and all subsequent mycologists could be badly confused by anyone who tried to interpret the Rules to the letter.

2. That Article 57 be rewritten somewhat as follows: *Among Ascomycetes and Basidiomycetes (but not Phycomycetes) with pleomorphic life-cycles, the first valid binary name applied to the perfect state of a species takes precedence. The names of imperfect states can still be used, and should be used if ambiguity might follow the use of the name of the perfect state.*

Thus, for example, mycologists now use, and it should be made legitimate for them to use, *Cladosporium herbarum* Fr. despite the fact that its perfect stage *Sphaerella Tulasnei* Jancz. has been found and verified. It may be misleading to cite the name of a perfect stage from a country where only the imperfect stage is known.

Those who may think Phycomycetes should bear the names (and, of course, the authorities) used when the perfect state (oospore or zygospore) of each species was first described should try to provide author citations for

Phytophthora infestans, other Peronosporales, and the zygomycetes. Article 5, in my opinion, excludes Art. 57 from applying to Phycomycetes; for the consequences of such application are more than doubtful, and it is established custom to use the first valid name applied, whether to imperfect or perfect state.—G. R. BISBY, Imperial Mycological Institute, Kew, Surrey, England.

BOOK REVIEWS

HOLTON, CHARLES STEWART, and FREDERICK DEFOREST HEALD. *Bunt or Stinking Smut of Wheat.* 211 p., 21 figs., Burgess Publishing Co., Minneapolis, Minnesota (U.S.A.) 1941. $3.25.

In this book the authors have summarized and briefly discussed most of the important investigations that have been reported on bunt and its causal organisms. The authors have fulfilled the purpose which they claim for the volume ". . . to make available a single publication that will serve as a source of general information on the present status of the bunt problem in its various aspects." The reviewer feels that it is fortunate that two men as well qualified for analyzing work with bunt as Holton and Heald undertook to bring together this information from its diverse sources and give us the benefit of their interpretations. They have produced a reference work with an almost complete bibliography and a resumé of what has been accomplished by the investigations which have been made with bunt. The book is in mimeoprint and is illustrated with 21 plates of well chosen material.

The book is divided into 11 chapters, each dealing with a separate phase of the bunt problem. The chapter headings in abbreviated form are: I. Introduction, II. Economic Importance, II. Species Distinction, Spore Germination, and Artificial Culturing, IV. Host Range, V. Factors Affecting Infection and Development, VI. Effect of Bunt on Morphology and Physiology of the Wheat Plant, VII. Physiologic Specialization, VIII. Cytology, IX. Heterothallism, Hybridization and Species Association, X. Varietal Reaction and Genetics of Resistance, and XI. Control Measures. In each chapter the authors have abstracted and discussed the papers they considered as giving the most important contributions to that particular phase of the problem. After reviewing the literature they have, in most chapters, summarized the results and pointed out the accomplishments, as well as the instances where fundamental information is lacking. These summaries and critiques add much to the value of the book. For each chapter a bibliography is given, and these include many references not cited in the text. There are more than 1100 references listed, 635 of these in Chapter XI on Control Measures.

A summary of the seed treatments that appeared to have been most effective and practicable in controlling bunt would have added materially to Chapter XI. Only a few errors are evident in the printing. Chapters IV, VII, and X should be of particular interest to plant breeders, as well as plant pathologists.—R. H. BAMBERG, Bureau of Plant Industry, U. S. Dept. of Agriculture.

GUILLIERMOND, ALEXANDRE. *The Cytoplasm of the Plant Cell.* Chronica Botanica Co., Waltham, Mass., 1941, 241 p., 152 figs.

Studies on damaged and destroyed cells, important as they are, reveal little of the nature of cellular organization, in its relation to metabolism, hence the limitations of physiological researches conducted on tissue extracts, *breis.* . . . One of the most variable respiratory systems of the cell, that which has been studied by Warburg, Keilin, and recently by Woods and DuBuy, seems closely bound to cell structure and greatly affected by alterations of that structure.

As the chemist has to know of the structure of molecules, so the biochemist has to know how molecules link up into the cellular structure, as perceptible through cytological observation.

To correlate the expression of disease with unbalanced cell metabolism, the pathologist has to interpret such changes in the cell structure as he can detect through direct microscopic observation of living tissues, or through the use of cytological or cytophysiological methods; from cytological data he can obtain information on the shifting of the balance between the various enzymatic systems on the interplay of which cell metabolism depends.

Guilliermond's exposition of the physical properties of the cytoplasm, of its chemical constituents and physico-chemical constitution, of the plastids and mitochondria and of their relationship, of the vacuoles, and lastly, of fatty degeneration and cytoplasmic alterations, lead us, through the use of a "convergent technique" to "seek the relationship between morphological structure and the physiological activity of the cell." The "cytologist must become both a chemist and a physicist," he "must at the same time remain a morphologist."—J. DUFRENOY, Louisiana State University, Baton Rouge, Louisiana.

REPORT OF THE 1942 ANNUAL MEETING OF THE SOUTHERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 1942 annual meeting of the Southern Division of The American Phytopathological Society was held in part as a section at the meeting of the Association of Southern Agricultural Workers, February 4, 5, 6, in Memphis, Tennessee. A joint session was held with the Agronomy Section on diseases of forage crops. About 50 plant pathologists attended the meeting, and approximately 12 formal papers were given. The fact that the meetings of The American Phytopathological Society were held in Dallas, Texas, in late December, 1941, explains the limited number of papers presented at Memphis in February 1942. A short business session was held on the morning of February 5, when the following officers were elected.

President, S. G. Lehman
Vice-President, W. H. Tharp
Counselor, A. G. Plakidas

Titles and abstracts of papers presented at the meeting follow.

I. L. FORBES
Secretary-Treasurer

Breeding Tobacco for Black-root-rot Resistance. HENDERSON, R. G. Breeding tobacco for resistance to black root-rot (*Thielaviopsis basicola*) has been carried on for the past 8 years. Crosses have been made between a root-rot-resistant variety of Turkish (Xanthia) tobacco and susceptible flue-cured and dark fire-cured varieties. The F_1 plants were larger than the Turkish parent but resembled it in most other respects. They were resistant to root rot, but the degree of resistance was not determined. The F_2 plants were extremely variable in leaf size and shape, petiole length, number of leaves, etc.; otherwise, they were intermediate in appearance between the two parents with closer resemblance to the Turkish parent. There was a noticeable difference in degree of resistance in the F_2 population. Selections in the F_2 and F_3 populations gave lines highly resistant to root rot. The best of these lines, however, closely resembled the Turkish parent, although some advance was made in obtaining a resistant plant of desirable type. Selections from the F_2, F_3, and later generations were backcrossed with the susceptible parent, but resistance was maintained more consistently at a high level when F_3, or later generations, were used. Selections in the progeny of the backcrosses gave resistant plants resembling the susceptible parent in general appearance. The leaves of the most resistant lines, however, were still small. A second backcross was required before plants with full-sized leaves were obtained.

In 1941 several resistant hybrids of the flue-cured type were tested on soil infested with *Thielaviopsis basicola.* One of these, No. 38, was highly resistant to root rot and appeared to have other desirable characteristics. The average height of the plants of this hybrid on August 11 was 74.4 in., while that of the adjacent check (Cash) plants was 37.5 in. Hybrid No. 38 also was included in the variety test at Chatham, Virginia, where root rot was not a factor. The hybrid produced 1264 lb. of cured tobacco per acre, valued at $420.80, as compared to the check (Yellow Mammoth), which yielded 1072 lb. per A., valued at $328.80.

The growth rate of the most resistant hybrids, as determined by height measurements, progressed at a uniform rate from the time the plants were well established in the field until they reached full height; whereas that of susceptible plants was slower during the first 2 months of the growing season than during the last month, indicating that the susceptible plants were stunted by root rot during the cooler part of the season.

Studies on Soil Sterilization with Urea and Calcium Cyanamid. HENDERSON, R. G. Heavy urea and calcium cyanamid applications added to tobacco plant-bed soil have given promising results in weed control. These chemicals produce marked changes in the physical structure, ammonia- and nitrate-nitrogen content, microbial population, pH, and O R potential of the soil. The time required for the urea and calcium cyanamid to be completely ammonified in the soil varies with the soil type and certain environmental factors. Manure and blackstrap molasses, applied with the urea, appear to increase nitrification. The ammonia concentration also apparently decreases more rapidly in plots receiving manure and blackstrap molasses. In an experiment conducted in the greenhouse on Granville type soil, about 60 per cent of the urea-nitrogen applied escaped from the soil as ammonia during a period of 5 months. Ground wheat straw, added to the soil with the urea, reduced this loss to about 33 per cent. The loss of ammonia-nitrogen from calcium cyanamid-treated soil in the same test was less than 9 per cent without straw and

only 2.4 per cent with straw. Platinum electrodes were buried in treated and nontreated soil in the greenhouse and, at frequent intervals, millivolt readings were taken from them with a calomel-half cell as a reference. While these readings may not represent the true Eh of the soil, they indicate the extreme change brought about by the heavy application of urea and calcium cyanamid. The millivolt reading dropped from more than 400 to about 50 following the treatment and rose very slowly until at the end of 6 months it was approximately 200. Straw added to the calcium cyanamid caused the reading, following the initial depression, to rise above 300 mv. at the end of the first month; but there was a gradual decline after that time, until it approximately equalled that without straw at the end of 6 months. The drop in E.M.F., due to the rise in pH, would be expected not to exceed 180 mv. On this basis it is evident that these treatments brought about a substantial change in the O R potential.

Oospore Production in Cabbage Seedlings by Peronospora parasitica. LEBEAU, F. J., AND J. A. PINCKARD. Oospores of *P. parasitica* proved abundant in the cotyledons and sparse in the true leaves of field-grown cabbage seedlings in south-central Mississippi. Oospore formation also appeared related to moisture, temperature, and light intensity, since the spores were most frequently observed in the tissues of seedlings in dense stands on the south side of wood-inclosed growing-frames during November and December. Most abundant field production of oospores occurred during rainy periods, which permitted fungal assimilation of food materials from invaded tissues. Dry, bright weather appeared to interrupt oospore formation because of desiccation of invaded tissues. Greenhouse-grown cabbage seedlings surrounded with cheesecloth produced abundant oospores approximately 15 days after inoculation, provided water was continuously atomized into the air above the plants. In view of these observations, the writers conclude that the life cycle of the cabbage downy-mildew fungus is completed with the formation of oospores, largely in the cotyledons, and that over-summering of the fungus is probably by means of oospores rather than by perennial mycelium in weed hosts.

Cotton-seed Treatment with Dust Preparations Containing Hormones Alone and in Combination with Ceresan and Spergon. LEHMAN, S. G. Cotton seed, before planting, was dusted in the field with two hormone preparations, one containing indolbutyricacid, the other potassium naphthalene acetate. Each hormone dust was used alone and in combination with Spergon in 2 tests and with 2 per cent Ceresan in 2 tests. The application rate or chemical seed ratio by weight was 1 to 113,000 for hormones, 1 to 480 for Spergon, and 1 to 160 for Ceresan. Each treatment was planted in a plot of 1 or 3 50-ft. rows in each replication, four replications being planted in each test. Neither hormone produced any observable effect on the number of seedlings emerged nor on the yield of seed cotton. Spergon increased the number of seedlings 11 per cent in one test and 22 per cent in another, both increases being highly significant statistically. Yield of seed cotton increased 6.2 per cent with Spergon in the one test for which yield records were kept. Ceresan gave no significant increase in number of seedlings emerged nor in yield. The tests with Spergon and Ceresan were planted at different dates, in different locations and with different seed varieties.

Results from the B-2 Regional Test with Reginned and Acid-delinted Cotton Seed. LEHMAN, S. G., D. M. SIMPSON, C. H. ROGERS, J. A. PINCKARD, AND C. H. ARNDT. The purpose of this test was to note the effect of removing the fuzzy or short lint from cotton seed before planting. Sublots, treatments, and per cent of lint on the seed were as follows: F, fuzzy (natural) seed, 14.9 per cent lint; R1, reginned, light cut, 8.1 per cent lint; R2, reginned, medium cut, 5.9 per cent lint; R3, reginned, heavy cut, 4.0 per cent lint; A, delinted with sulphuric acid, no lint; AS, delinted with sulphuric acid and subsequently scarified. Following the primary treatments each sublot was divided into 2 aliquots, one of which was dusted with 5 per cent ethyl mercury phosphate dust (New Improved Ceresan) and the other left undusted. Six plantings of all sublots were made in North Carolina, 2 in Tennessee, 1 in Mississippi and 1 in Texas. Three plantings of the Ceresan-treated aliquots were made in South Carolina. In the 6 plantings in North Carolina, the mean percentage increase in number of seedlings at the last count before thinning for each primary treatment above the natural fuzzy sublot was as follows: R1, 13; R2, 22; R3, 23; A, 53; and AS, 48 per cent. Increase for Ceresan dusting was 13 per cent. For the 10 plantings in N. C., Tenn., Miss., and Texas the corresponding percentage increases were as follows: R1, 8; R2, 14; R3, 16; A, 41; and As, 35 per cent; and for Ceresan dusting, 16 per cent. For the 3 plantings in South Carolina, where only the Ceresan dusted aliquots were used, the following percentage increases were obtained; R1, 64; R2, 51; R3, 47; and A, 61 per cent. All of the increases reported above were highly significant statistically. All reginned and all acid-delinted sublots emerged more rapidly than the sublots with the normal amount of lint.

Seed-treatment Studies of Vegetable and Ornamental Plants. PERSON, L. H. AND S. J. P. CHILTON. Tests were made of cuprocide, red copper oxide, yellow copper oxide, zinc oxide, Vasco 4, Ceresan, and Semesan as seed treatments, and yellow copper oxide, zinc oxide, Vasco 4, Leafox 200, formaldehyde dust, and sand as soil treatments for tomatoes, peppers, and eggplants. Seeds treated with the copper dusts gave the best results over a period of 3 years. Semesan and Ceresan were sometimes toxic, and the zinc compounds gave erratic results at times when used on the seed. The copper dusts in water and the zinc compounds dusted on the surface were of equal value as soil treatments. Formaldehyde dust as a soil treatment was of little value. Sand reduced emergence approximately 10 per cent. With tomatoes and peppers, seed treatment with cuprocide, Merck's red copper oxide, or yellow copper oxide was usually sufficient to obtain good stands without soil treatment. With eggplants, seed treatment with the copper dusts, and soil treatment, either with a water suspension of the copper compounds or a covering of one of the zinc compounds given above, was necessary to insure good stands.

Cuprocide, yellow copper oxide, Vasco 4, Leafox 200a, 1286a, 1286ecc, Spergon, Barbak C, Ceresan, New Improved Ceresan, and New Improved Semesan, Jr., were tested on the seed of various ornamental plants in the greenhouse. The mercury dusts were toxic where applied full strength. Yellow copper oxide gave the best results of all compounds tried on centaurea, zinnia, cosmos, calendula, and pansies.

The Effect of Depth of Planting on Fuzzy and Acid-delinted Cotton Seed. RAY, W. WINFIELD. Field experiments were made with Ceresan-treated fuzzy and acid-delinted seed in which 4 depths of planting, 1, 1¾, 2½, and 3¼ inches, at 2 rates and 2 dates were employed. The data for both stands and yields were analyzed by the analysis-of-variance method. The delinted seed proved superior statistically to the fuzzy seed at the 4 depths of planting. No significant differences existed between the 2 shallower depths of planting for either of the 2 seed types, but both of the shallower depths of planting gave stands and yields statistically superior to those of the 2 deeper plantings. It is concluded that the depth of planting normally employed for fuzzy seed is favorable for acid-delinted seed and that the acid-delinted seed gives stands and yields superior to those from fuzzy seed.

Results of Seed Treatment Tests on Peanuts. SHAW, LUTHER. Nineteen chemical dusts were extensively tested in the summer of 1941 to determine their effects on emergence of peanuts when seed-dusted before planting. Results of 3 suitably replicated tests, conducted in the field, show an average emergence of 23.2 per cent for the nontreated plots. One organic sulphur and 2 organic mercury materials consistently gave the greatest emergence, averaging 65.5 per cent for the organic sulphur, and 63.0 and 60.4 per cent for the mercury materials. Spergon, and a 20–80 mixture of lime and sulphur resulted in emergences of 59.4 and 52.9 per cent, respectively. Eight copper compounds were used, the copper content of each dust having been adjusted to 10 per cent with talc as a diluent. All of these materials, excepting one, gave increased emergences of medium rank. Most of the other materials tested gave small increases in emergence. Observations and preliminary experiments indicated that the increases in emergence obtained from seed treatment resulted from prevention of seed decay prior to germination. It was also evident that most of this decay took place during the first 3 to 4 days after planting.

Results of Preliminary Experiments on the Control of Root Diseases of the Peach. SHAW, LUTHER. Results of preliminary experiments conducted at 2 locations in North Carolina in the 1941 season indicate that certain chemical soil treatments offer promising aid in replanting old orchard sites. One of the locations involved an area planted to Elberta peaches for 4 years, and the other an area planted to the same variety for about 20 years. An examination of trees pulled prior to undertaking the experiment revealed the presence of root knot, crown gall, and root rots at both locations, the rots being prominent at the older site only. Bands of soil 6 feet wide, centering on the tree row and extending through 5 old tree sites received varying rates of the following 10 treatments: Chloropicrin, Paradichlorobenzene, benzol, sulphur, cyanamide, urea, basic slag, nitrate of soda, potash, calcium hypochlorite, Spergon, and Spergon No. 98 special. About 2 months after treatment all plots were planted with Belle of Georgia peach trees on native root stock. Two additional nontreated plots were planted with the same variety as controls, and one with the same variety on Shalil root stock. Results of these tests show: (1) Shalil root stock was practically immune from root knot, but was susceptible to crown gall; (2) urea gave excellent disease control at both locations, but severely stunted the trees; (3) chloropicrin resulted in excellent tree survival and vigor at both locations, but gave only moderate root-knot control in the younger orchard site; (4) the remaining treatments gave little to no improvements in disease control or tree survival over that of the controls.

Further Studies on the Reaction of Commercial Cotton Varieties to Root-knot Nematode. SMITH, A. L. Reaction to root-knot of commercial varieties studied in 1941

confirm results previously reported (Phytopath. 31: 1099–1107, 1941). A system is described for expressing root-knot susceptibility in terms of a single figure, the "root-knot index." The range of values varies from 0 to 100, the latter term representing complete susceptibility. This system facilitates comparisons and permits statistical analysis of the data. A comparison of 25 commercial varieties shows Coker's 4 in 1–4 and Coker's 4 in 1–5 to be superior to others in root-knot resistance. Inbred lines selected from commercial varieties varied widely in resistance and indicate possibilities for improving root-knot resistance. Additional evidence was obtained suggesting that root-knot resistance is inherited recessively.

Root-knot Resistance of Five Soybean Varieties. TAYLOR, A. L. A method for testing resistance of plants to the root-knot nematode, *Heterodera marioni*, was devised and tested in 4 experiments at 3 locations on the Laredo, Biloxi, Otootan, Clemson, and Georgian varieties of soybeans. Irregularities in the results at the different locations suggest that root-knot resistance varies with ecological conditions. None of the tested varieties of soybeans was found resistant enough to warrant its recommendation for use in nematode-reducing rotations.

Chemical Control of Root Knot. TAYLOR, A. L. The root-knot nematode, *Heterodera marioni*, can be controlled in soil in the field or greenhouse by the use of chloropicrin, ethylene dichloride, carbon bisulphide or methyl bromide. All except methyl bromide are applied by injecting measured amounts into the soil at regular intervals. Methyl bromide is applied by releasing the gas under an air tight soil cover. Costs per acre, including an allowance for application, are: Chloropicrin, $180 to $360; ethylene dichloride, $340 to $360; carbon bisulphide, $70 to $90; methyl bromide, $150 to $200. Carbon bisulphide cannot be generally recommended because of its extreme fire and explosion hazard. Where crops are planted in widely spaced rows or hills, the "spot treatment" technique can be applied with chloropicrin at costs ranging from $8 to $10 per acre for watermelons to $35 to $40 per acre for tomatoes. Methyl bromide is particularly effective for treating potting soil, complete control of root knot being obtained with ½ ml. per cu. ft. and a cost of less than 1 cent per cu. yd. Ethyl mercury iodide also may be used for control of root knot on potting soil high in organic matter, but appears to be toxic to some species of plants.

Distribution of the Root-knot Nematode in High Ridge Plantings of Potatoes and Tomatoes. THORNE, GERALD. The root-knot nematode, *Heterodera marioni*, has a very definite behavior in the potato fields of western Nevada, where production is by irrigation. The water is applied through deep furrows between the rows. Most of the tubers are produced within ridges formed by "hilling up" the plants as the furrows are dug.

Irrigation water applied to *level* fields stands deep in the furrows and the soil is saturated, giving the nematodes every opportunity to reach plants, with the result that practically every tuber is heavily infected and unfit for use. Water flowing rapidly through the furrows of *sloping* fields rises in the ridges only by capillary action and the soil does not become saturated. Nemic activity is thereby restricted to the lower portions of the ridges, and tubers from the top 4 or 5 inches are generally clean. Below these uninfected tubers a few nematodes appear and the numbers increase with the depth until, near the water level in the furrows, the tubers are severely infected.

Similar conditions were observed in 2 California tomato fields, one near Santa Ana, the other near Modesto. In both instances frequent, shallow irrigation between high ridges produced good crops. Downward growing roots were soon clubbed and deformed by root knot, but those growing laterally made a remarkable growth, often extending 6 to 10 ft. along the ridges with little or no infection. The owner of the Santa Ana field stated that filling the furrows with water just once would destroy the crop within a week.

Moisture appears to be the principal underlying factor in producing the above modes of root-knot infection, since temperatures and soil types were very different in the 2 widely separated localities observed.

Populations of Root-knot Nematode Larvae in Two Kern County, California, Fields. THORNE, GERALD, MERLIN W. ALLEN, JAMES HARE, and MARK A. LINDSAY. A preliminary survey of Kern County, California, in the summer of 1941, showed upwards of 75,000 acres of land infested with the root-knot nematode, *Heterodera marioni*. Two severely infested fields were selected for study, one near Arvin, the other near Shafter. Soil samples were taken in 6-inch columnar sections to a depth of 5 feet and examined for root-knot larvae by the Cobb sifting and gravity methods. Great variation was found in the numbers of larvae in the various sections and between the total samples. Distribution of larvae appears determinable by location of roots of cotton and alfalfa grown in the fields for 3 preceding years. Highest populations of individual samples varied from the 7- to 12- to the 55- to 60-inch sections, usually being in, or below, the 19- to 24-inch section. Most samples failed to reach the extreme depths occupied by the larvae, in some

instances the maximum population was in the 55- to 60-inch section, indicating that larvae doubtless would have been found deeper had the samples been taken.

Young roots of alfalfa, following cotton, were rarely attacked, although they were closely associated with thousands of larvae. The reason for this failure to make a ready transfer of hosts is problematical but it may be due to the existence of 2 distinct strains or races of *H. marioni* or to the host preference development when either crop is grown continuously for a number of years.

These studies emphasized the futility of chemical treatment of the soil following alfalfa or cotton in this locality, because of the extreme depths at which a major portion of the nematode population is located. Added evidence is given to the necessity of evaluating host plants in a given locality when planning crop rotations for nemic control.

Cotton-seed Treatments and Angular-leaf-spot Control. YOUNG, V. H. A study involving the Acala variety of cotton was made to determine the effects of sulphuric acid delinting of cotton seed and of flotation grading of the delinted seed into "floaters" and "sinkers," combined with the use of New Improved Ceresan on incidence of cotton diseases, percentages of emergence and yields of seed cotton. The following six treatments were employed: Fuzzy seed, nontreated (check); fuzzy seed treated with Ceresan; acid-delinted "floaters" nontreated; acid-delinted "floaters" treated with Ceresan; acid-delinted "sinkers" nontreated; acid-delinted "sinkers" treated with Ceresan. All treatments greatly reduced the incidence of seedling diseases in comparison to the fuzzy nontreated checks, but no other treatment was superior to fuzzy seed treated with Ceresan in that respect. Counts made September 1 showed 75 per cent of plants in nontreated check plots affected with angular leaf spot (*Bacterium malvacearum*) compared to 13 to 35 per cent in other plots. Emergence was little affected by any of the treatments employed. Flotation grading in this particular case failed to increase the percentage of emergence. Yields for all plots planted with treated seed were 31 to 48 per cent greater than for plots planted to fuzzy nontreated seed, but differences between treatments were not statistically significant. Data were analyzed statistically by the analysis-of-variance method. Under the conditions of this experiment, all treatments gave highly significant yield increases over nontreated checks and highly signficant decreases in the incidence of seedling blights and angular-leaf-spot infections, compared to the checks, but acid delinting and flotation grading failed to give significantly better results than were obtained from the use of ethyl mercury phosphate dust on normal fuzzy seed.